FWHM (seconds) boxplots of peptide peak widths and unique protein identifications from 80ng Hela tryptic digest across 50 × 5min gradient runs.

5cm X 150μm  180 samples per day
Aurora series column with a timsTOF Pro.

FWHM (seconds) boxplots of peptide peak widths and unique protein identifications from 200ng Hela tryptic digest across 50 × 17min gradient runs.

15cm X 75μm  50 samples per day
Aurora series column with a timsTOF Pro.

Nothing comes close.
Simplified high-throughput methods for deep proteome analysis on the timsTOF Pro.

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Recent advances in mass spectrometry technology have seen remarkable increases in proteomic sequencing speed, while improvements to dynamic range have remained limited.

An exemplar of this is the new timsTOF Pro instrument, which thanks to its trapped ion mobility, pushes effective fragmentation rates beyond 100 Hz and provides accurate CCS values as well as impressive sensitivity.

Established data dependent methodologies underutilize these advances by relying on long analytical columns and extended LC gradients to achieve comprehensive proteome coverage from biological samples.

Here we describe the implementation of methods for short packed emitter columns that fully utilize instrument speed and CCS values by combining rapid generation of deep peptide libraries with enhanced matching of single shot data dependent sample analysis.

Impressively, with only a 17 minute gradient separation (50 samples per day), the combination of high performance chromatography and CCS enhanced library based matching resulted in an average of 5,931 protein identifications within individual samples, and 7,244 proteins cumulatively across replicates from HeLa cell tryptic digests.

Additionally, an ultra-high throughput setup utilizing 5 min gradients (180 samples per day) yielded an average of 3,666 protein identifications within individual samples and 4,659 proteins cumulatively across replicates.

These workflows are simple to implement on available technology and do not require complex software solutions or custom made consumables to achieve high throughput and deep proteome analysis from biological samples.