Turnover optimized short nanoLC gradients on a tims equipped QTOF for high throughput and deep proteome measurements

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Introduction

High sample throughput in proteomics, similar to that in genomics, is highly desirable. Moreover, the highest analytical depth in proteomics is only achieved on fractionated samples, requiring subsequent analysis with short gradients to achieve reasonable overall measurement times per sample. The timsTOF Pro with trapped ion mobility spectrometry (TIMS) offers additional separation power and increased peak capacity. The powerful Parallel Accumulation Serial Fragmentation (PASEF) method (Meier et al., JPR 2015) for very high sequencing speed is perfectly suited for proteome analysis on short gradients. We have optimized MS conditions, column lengths and LC overhead times to obtain runs of 28.8 min injection to injection (50 samples/day) on the nanoElute (Bruker Daltonik) and demonstrate applicability for high throughput application.

Methods

HeLa cells and murine cerebellum were digested with trypsin. Peptides (250 ng) were separated for LC-MSMS with modified nanoLC method for short columns (Fig.1) including reduced overhead times (nanoElute, Bruker Daltonik). A 100 mm fritted column (PepSep, Denmark, packed with ReproSil 1.9 µm C18 beads, pore diameter 120Å, ID 75 µm) was connected to a zero dead volume emitter (PepSep, ID 20 µm fused silica emitter) providing high chromatographic resolution at a flow rate of 1 ul/min. A High-resolution timsTOF Pro mass spectrometer (Bruker Daltonik) utilizing the PASEF acquisition method was used. The PASEF cycle was set to 0.5 s equating to 100 ms TIMS MS scan followed by four 100 ms PASEF MS/MS cycles each fragmenting on average 12 precursors. For offline fractionation, 25 µg peptides were fractionated at high pH on reversed phase columns (Waters Acquity CSH C18 1.7 µm 1 × 150 mm). Data were analyzed using PEAKS 8.5 (Bioinformatics solution Inc.) and MaxQuant (Jürgen Cox, Max Planck Institute of Biochemistry). Results were filtered to 1% FDR.

Results

The high analysis speed of the timsTOF Pro and additional gas phase separation makes this instrument a perfect match for short gradient analysis. Reduced overhead time (Fig. 1B) shows both good gradient stability (Fig. 2A) and retention time reproducibility of peptides (Fig. 2B). More than 4200 Protein groups can be quantified with a median number of 4180 IDs across 30 runs (Fig. 2C). On the unique peptide level more than 26000 were identified (Fig. 2D). Chromatographic peak widths of 11.3s (median) are achieved on the 10 cm columns. The protein overlap between randomly selected runs is high (~90%) especially when taken into account that the runs are short and no matching between runs is applied (Fig. 2F). Same applies for peptides (Fig- 2G). For protein quantification very good correlation coefficients (Fig. 2F) and good protein CV values (Fig. 2I) can be achieved. Offline high pH reversed phase fractionation into 24 fractions allows deep proteome analysis of 9000 protein groups in HeLa and 10.000 IDs in murine cerebellum (Fig. 2J) in reasonable measurement time (<12h) and provides access to large scale investigations on peptide CCS values (Fig. 2K)

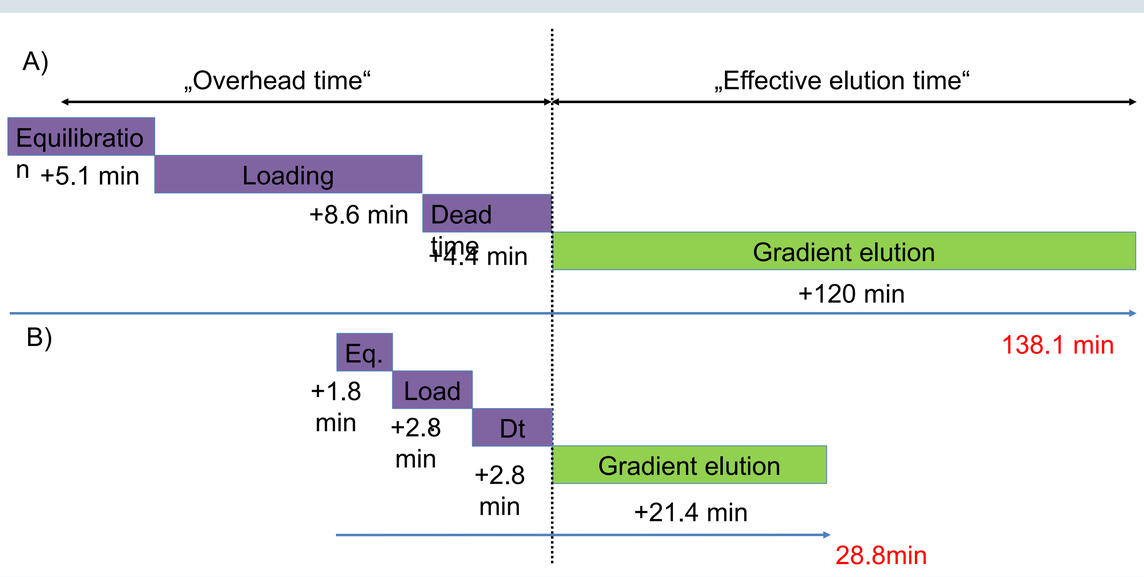

Figure 1: Overhead time reduction to enhance time effectiveness on short nanoLC-MSMS gradients

A) On usual 120min LC gradients overhead times from the end of a previous run to elution of the next run take 18 min which is acceptable on long runs. B) Overhead times were significantly reduced for short gradient elution by running at high flow rates and adjustment of equilibration steps. Reduced overhead times allow running 50 samples/day with efficient use of time for peptide elution.

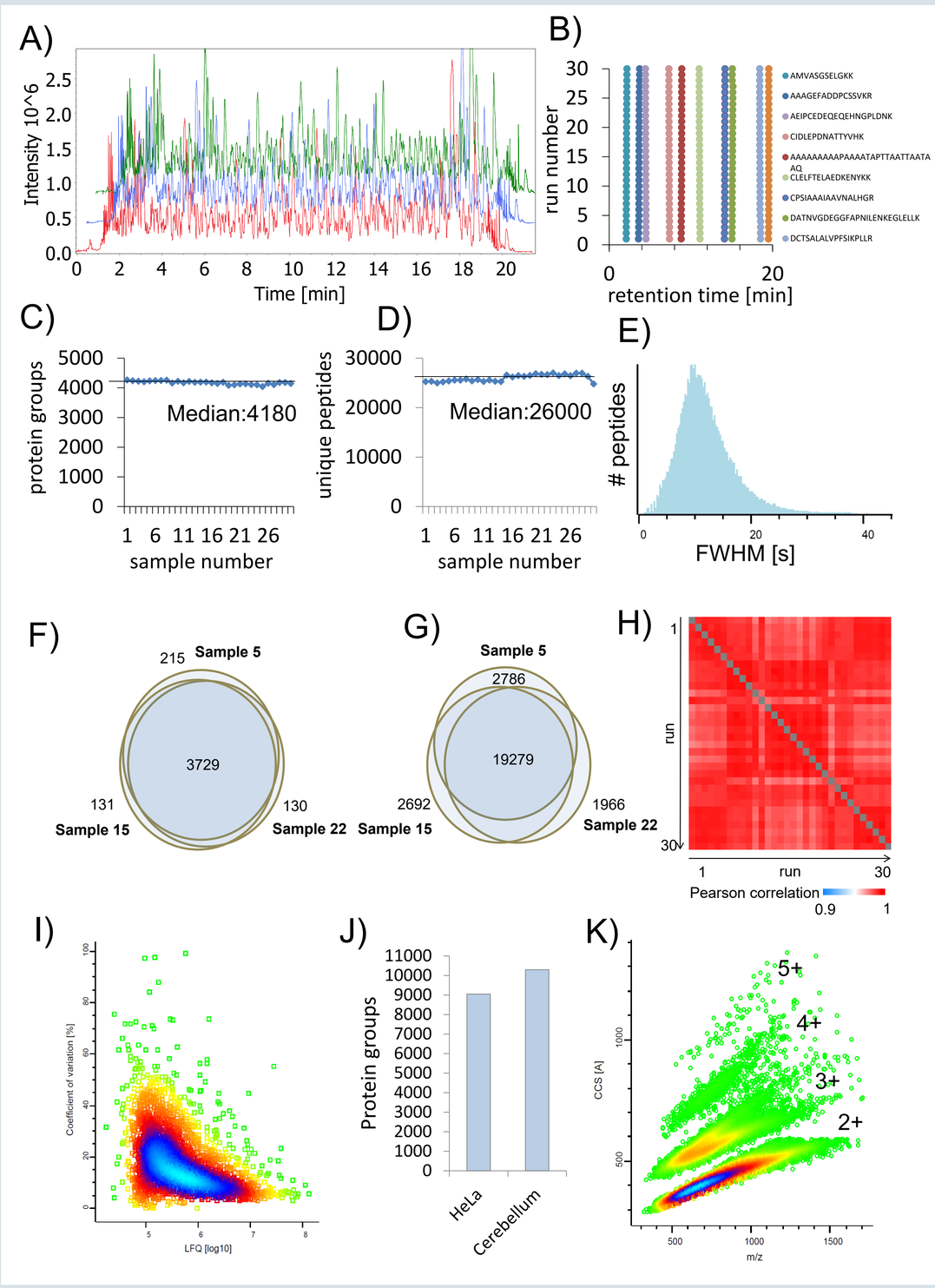

Figure 2: High reproducibility for running 50 samples/day and deep proteome analysis on fractionated samples

A) Reproducibility base peak chromatograms from 250 ng tryptic HeLa digest. B) High reproducibility on peptide retention times across 30 consecutive runs. C) 4180 Protein identifications and D) 26000 unique peptide identifications across 30 consecutive runs (1% FDR). E) Distribution of peak width (FWHM) of 10cm columns and 21.4 min gradient length. F) Overlap of protein IDs of three randomly selected runs and G) Overlap of unique peptides of the same runs. H) Correlation coefficients for LFQ quantification between 30 replicates. I) Coefficient of variation across 30 replicates for each single protein quantified in at least 3 conditions. J) High pH reversed phase fractionation into 24 fractions corresponding to <12h measurement time and identifications of >9000 protein groups from HeLa and >10000 protein groups from murine cerebellum. K) Large scale representation of 100.000 collisional cross sections from HeLa peptides.

Conclusions

- Reduced LC overhead times enable efficient use of MS instrument time
- Highly competitive ID numbers of 4200 Protein IDs at 50 samples/day by identification without matching to libraries
- High reproducibility between runs on protein/peptide IDs and quantitative values

timsTOF Pro