Short nanoLC gradients optimize throughput on a tims equipped QTOF with PASEF

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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 by LC-MSMS with a modified nanoLC method for short columns (Fig.1 A and B).

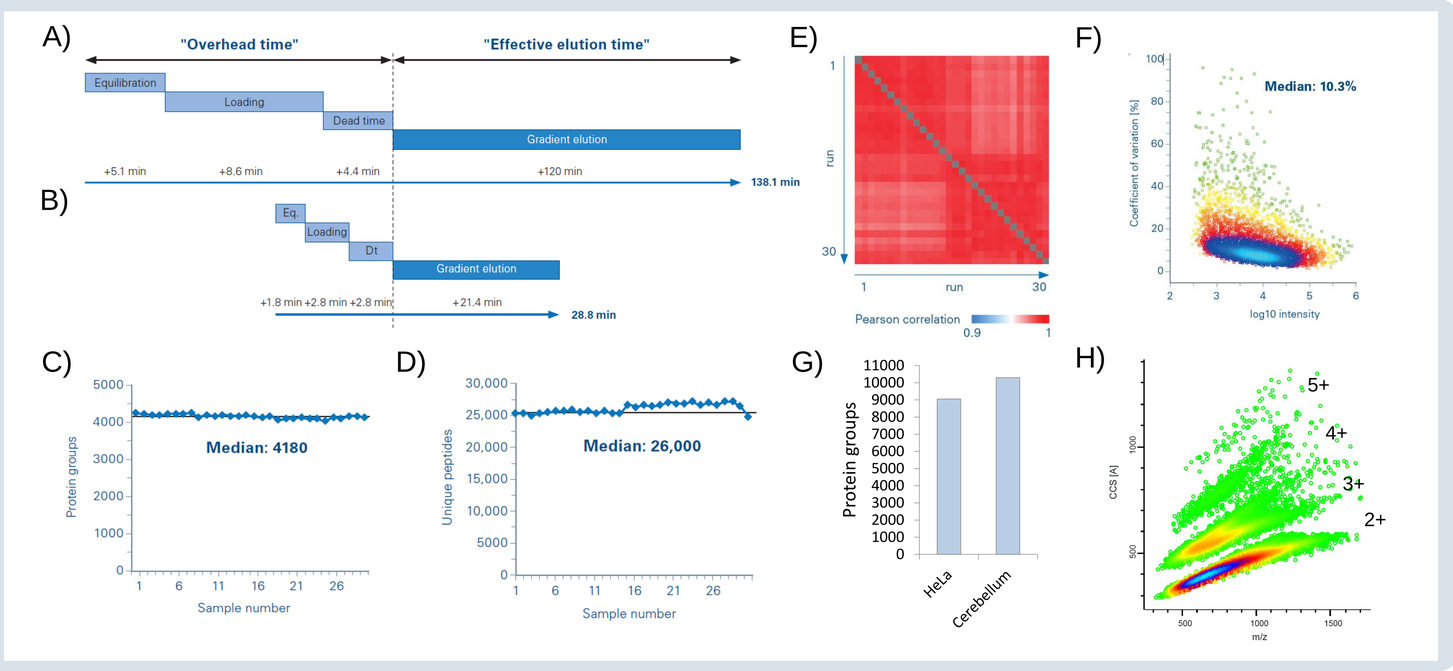

Figure 1: A) On usual 120min LC gradients overhead times from the end of a previous run to elution of the next run take 18 min which is acceptable on long runs. B) Overhead times were significantly reduced for short gradient elution by running at high flow rates and adjustment of equilibration steps. Reduced overhead times allow running 50 samples/day with efficient use of time for peptide elution. C) 4180 Protein identifications were consistently identified across 30 runs of 200ng HeLa corresponding to 26.000 unique peptide identifications D)(1% PSM FDR). E) Correlation coefficients for LFQ quantification between 30 replicates (R>0.97). F) Coefficient of variation across 30 replicates for each single protein quantified in at least 3 conditions. G) High pH reversed phase fractionation into 24 fractions corresponding to <12h measurement time and identifications of >9000 protein groups from HeLa and >10.000 protein groups from murine cerebellum. H) Large scale representation of 100.000 collisional cross sections from HeLa peptides.

A 100 mm fritted column (Bruker TEN) was connected to a zero dead volume emitter (20 µm fused silica emitter) providing high chromatographic resolution at a flow rates of 500 nl/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 and results were filtered to 1% PSM FDR.

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