Simplified high-throughput methods for deep and targeted proteome analysis on the timsTOF Pro

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Introduction

Established proteomic methodologies have relied on long analytical columns and extended UHPLC gradients to achieve comprehensive proteome coverage. The timsTOF Pro pushes effective fragmentation rates beyond 100Hz and provides accurate collisional cross section (CCS) where established methodologies underutilize these advances. High-throughput proteomics, a practice used for large-scale protein characterization, screening and expression is highly desirable but limited by gradient length and instrument sensitivity. In this study, we have developed novel LC-MS methods for packed emitter columns that fully utilize instrument speed and CCS values to enable the analysis of high numbers of protein expression profiles in less than a day.

Methods

HeLa digest (Pierce) and Neat plasma (in-house) were analysed to determine the performance, sensitivity and robustness of the novel methods. Samples were fractionated using RP-HpH fractionation in a stage-tip format (Figure 1). Samples were injected by nanoElute (Bruker) or M-class (Waters) UHPLC coupled online to a timsTOF Pro mass spectrometer (Bruker). Peptide separation was performed using (15 cm x 75 µm i.d.) or (5 cm x 150 µm i.d.) packed emitter columns, both packed with reversed-phase 1.6 µm C18 coated porous silica beads (Figure 3, Aurora Series, IonOpticks, Australia). A linear gradient was run for 17 min or 5 min, respectively (Figure 2). 4 dimentional (4D) peptide data was acquired using data-dependent PASEF before analysis using MaxQuant (Figure 1).

Benchmark rapid analysis workflows

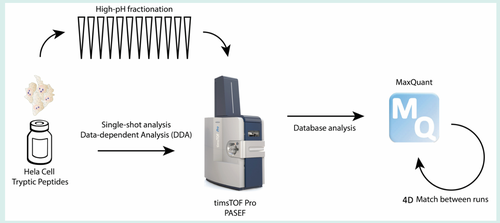
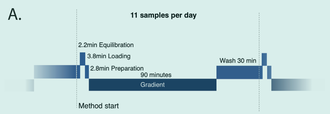
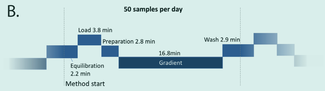


Figure 1. Schematic of experimental procedure

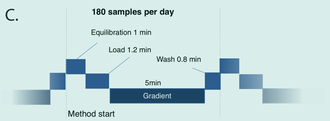
Optimised UHPLC methods



Column configuration: 15cm length X 75µm inner diameter, 1.6µm C18 resin.



Column configuration: 15cm length X 75µm inner diameter, 1.6µm C18 resin.



Column configuration: 5cm length X 150µm inner diameter, 1.6µm C18 resin.

Figure 2. Schematics of UHPLC methods. A) 90min sample gradient (11 samples per day). Total method time 128.8min. B) 16.8min sample gradient (50 samples per day). Total method time 28.5min. C) 5min sample gradient (180 samples per day). Total method time 8min.

IonOpticks Aurora Series Column

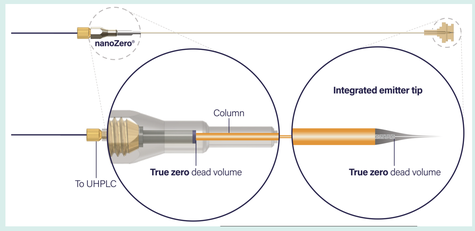


Figure 3. There are 2 key features that give IonOpticks Aurora columns robust performance with plug-and-play usability. The first is an integrated packed emitter tip for true zero dead volume to eliminate peak broadening post-column. The next feature is the nanoZero union which can receive a fitting from a UHPLC such as a nanoViper and can withstand pressures >1200bar. This fitting also features a unique design where the end of the nanoViper abuts the start of the column without a bore in-between resulting in a true zero dead volume union.

Results

Short gradients allow reproducible analysis of samples

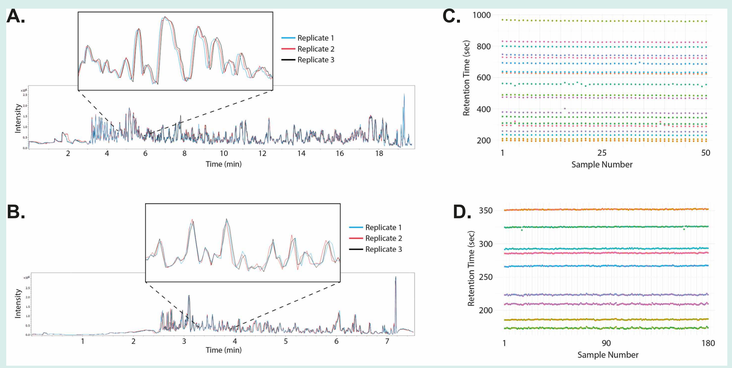


Figure 4. A) and B) Comparison of three base peak chromatograms from a HeLa tryptic digest (200ng injection) for the 17 (A) and 5 (B) minute gradients. C) and D) Retention time stability of selected peptides (one colour per peptide) from 200ng (17min, 50 samples) or 80ng (5min, 180 samples) injections of a Hela tryptic digest that were identified across all samples.

Peak characteristics from short gradient methods

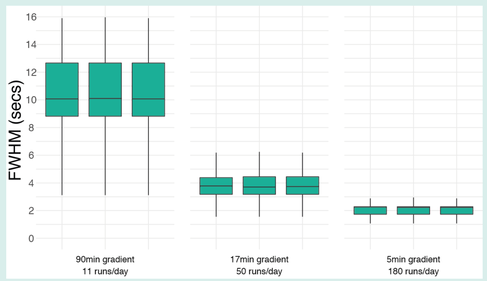


Figure 5. Box plot of identified peptides full width at half maximum (FWHM) in seconds for column length and gradient duration combinations (see figure 2). Three replicates of 200ng Hela tryptic digest injections shown. Line indicates median FWHM for each sample.

High number of protein IDs achieved from short gradients

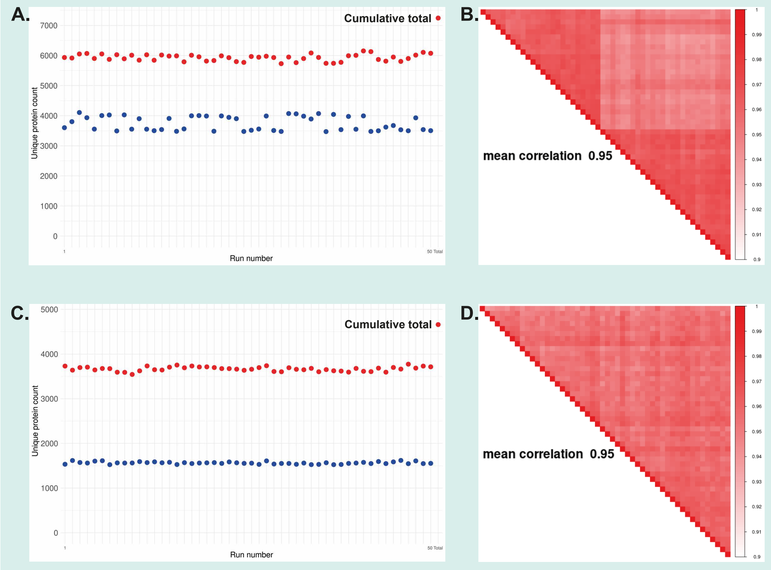


Figure 6. Unique protein group identifications and quantitative correlation from Hela tryptic digest using 50 (A. and B. ; 17min gradient; 200ng injection) and 180 (C. and D. ; 5min gradient; 80ng injection) samples per day single shot runs. Single shot samples were matched to 12 high pH reversed phase fractions of the same samples.

Short gradients facilitate analysis of Plasma samples

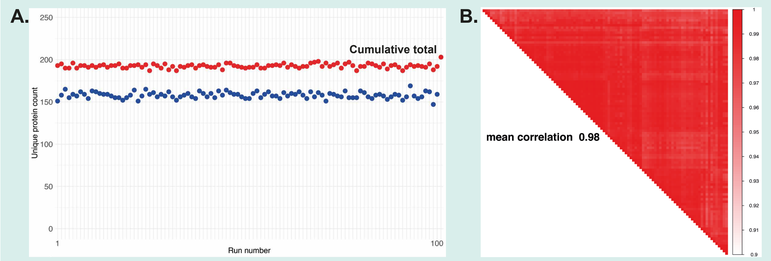


Figure 7. Unique protein group identifications (A) and quantitative correlation (B) from neat Plasma digest using 180 (5min gradient; 50ng injection) samples per day single shot runs.

Conclusions and references

The described 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.

Simplified high-throughput methods for deep proteome analysis on the timsTOF Pro. ***bioRxiv*****.** 2019 Jun 3. JJ Sandow, Infusini G, Dagley LF, Larsen RH, Webb AI