

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

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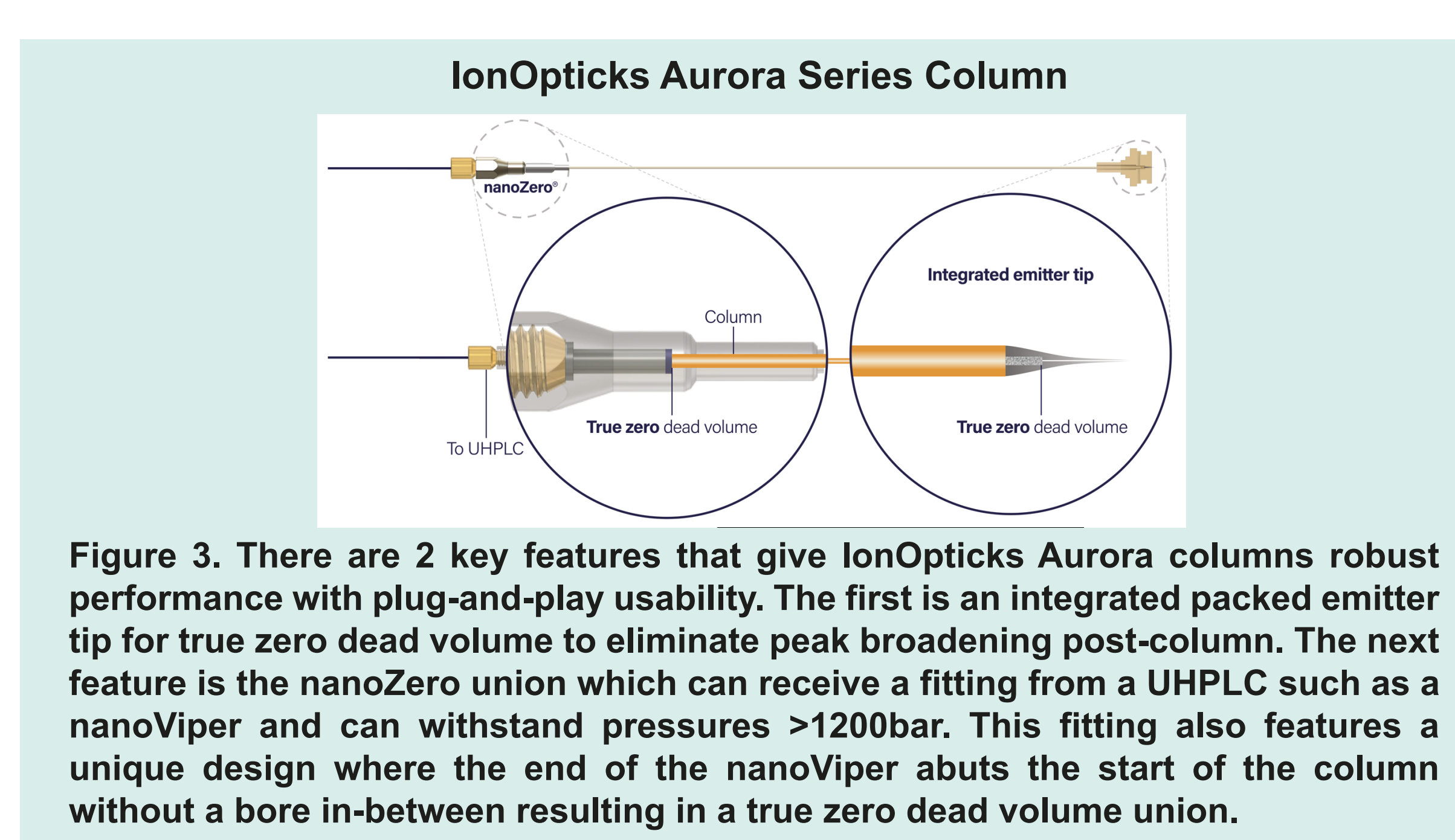
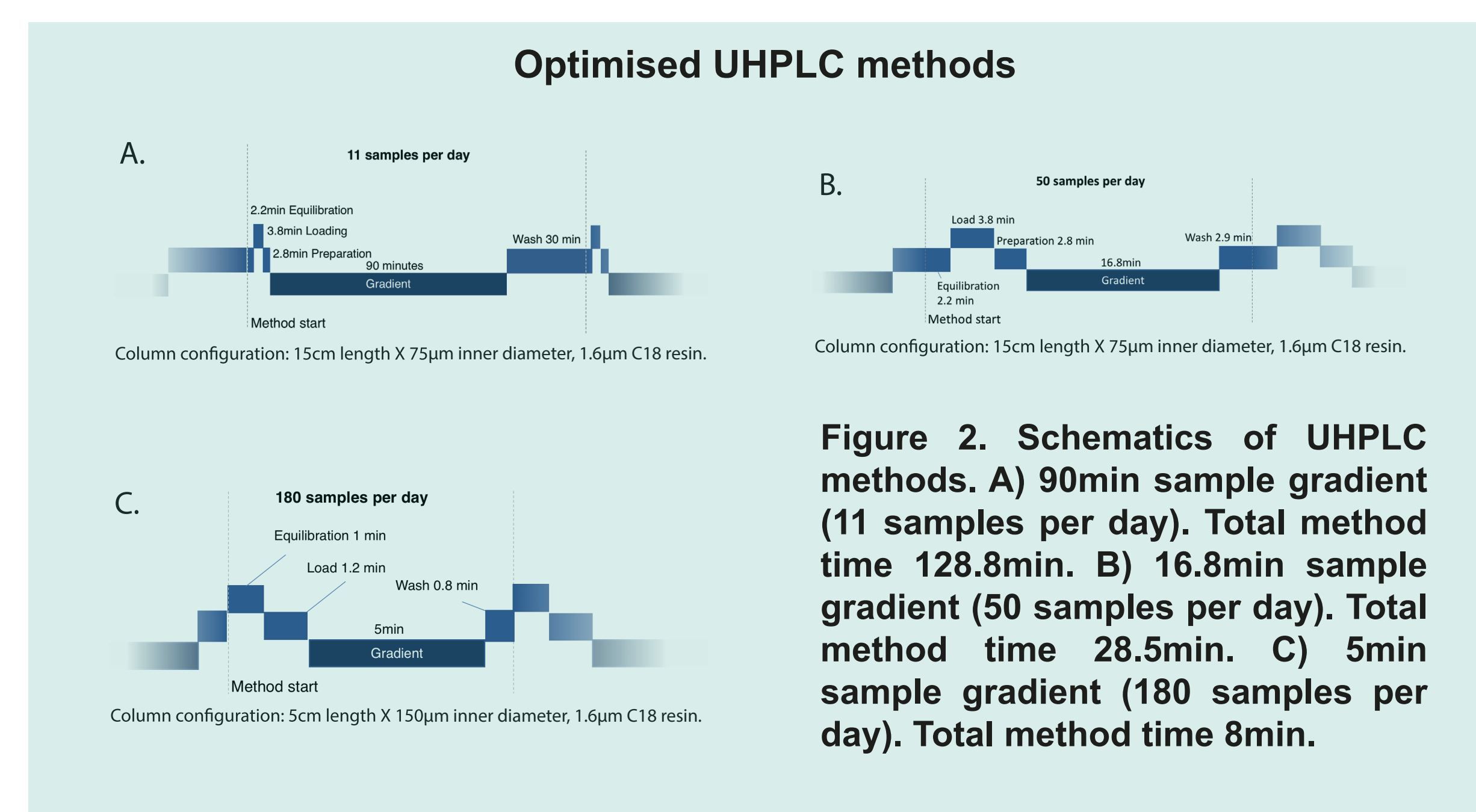
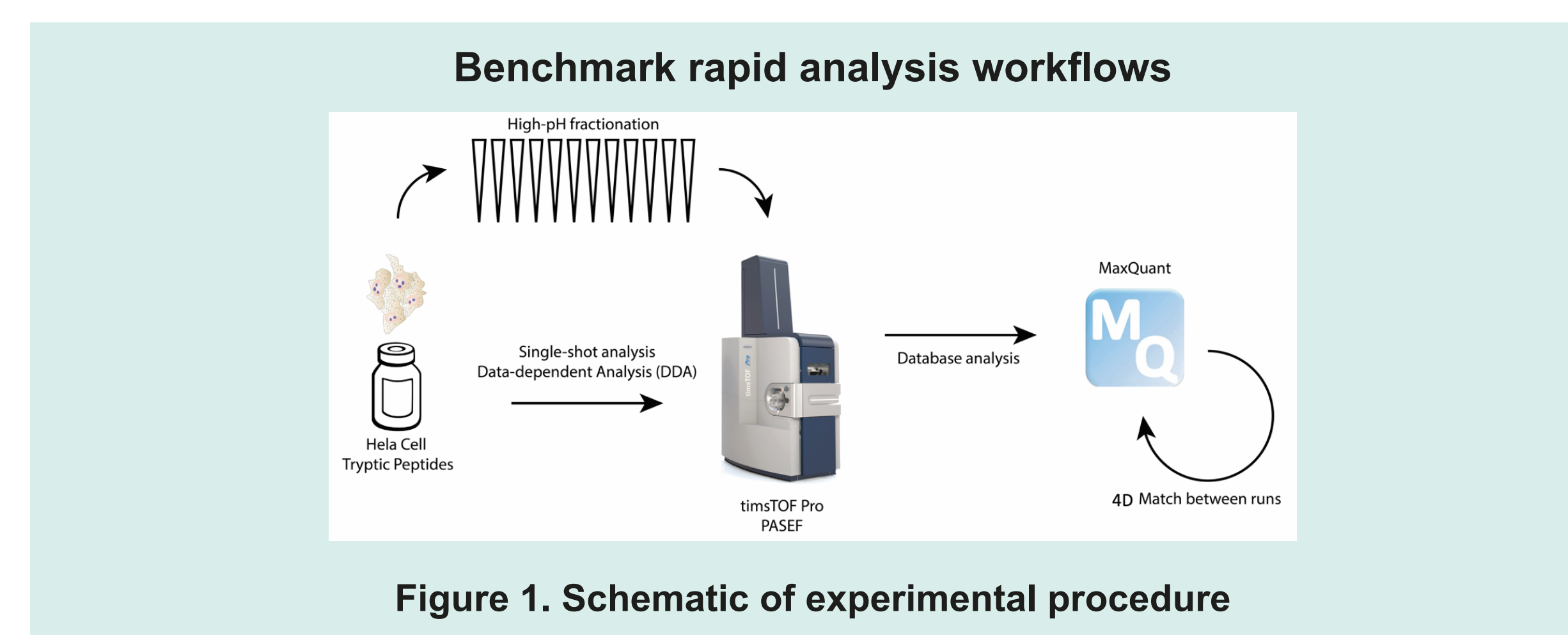
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## Introduction

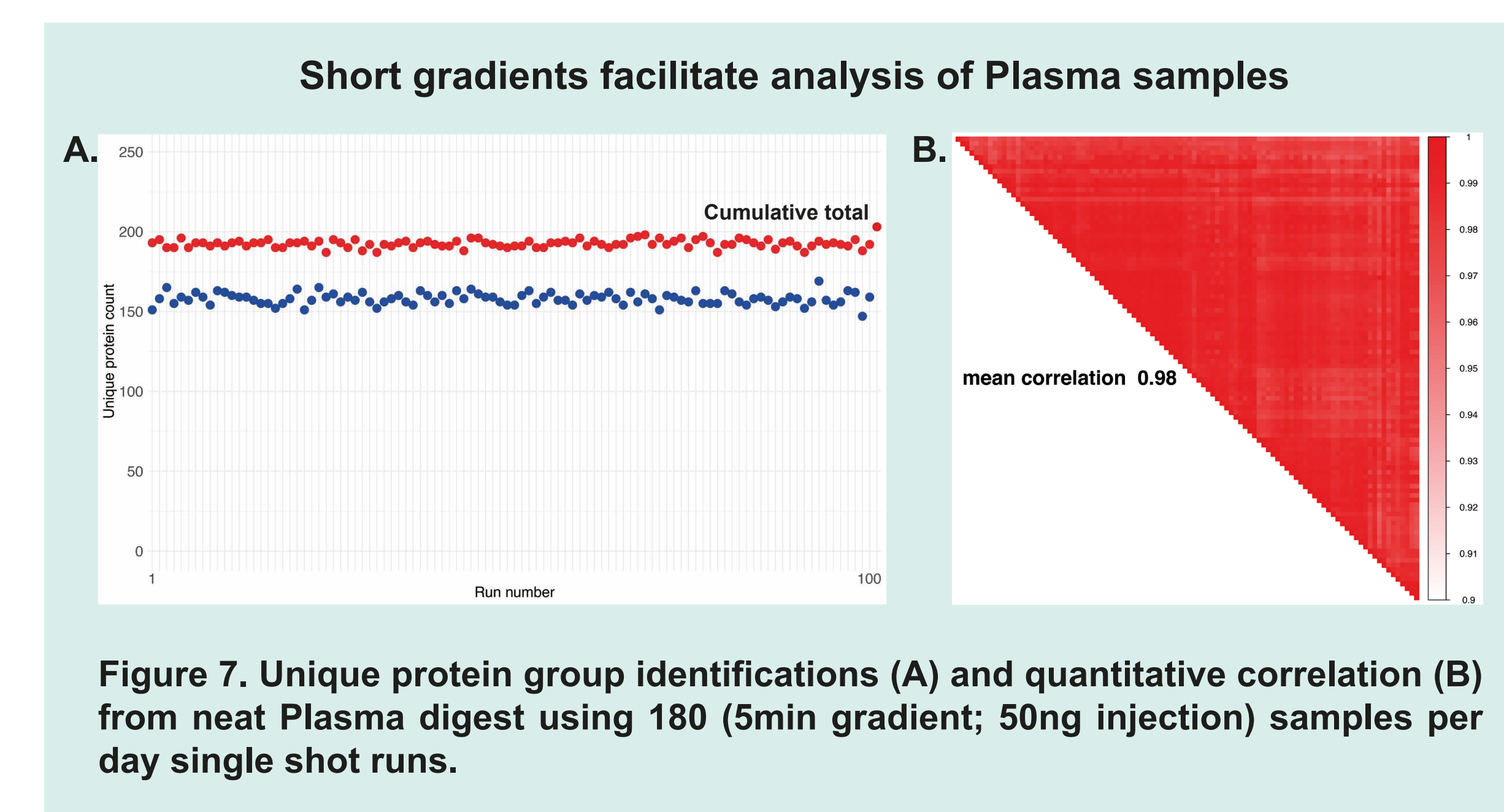
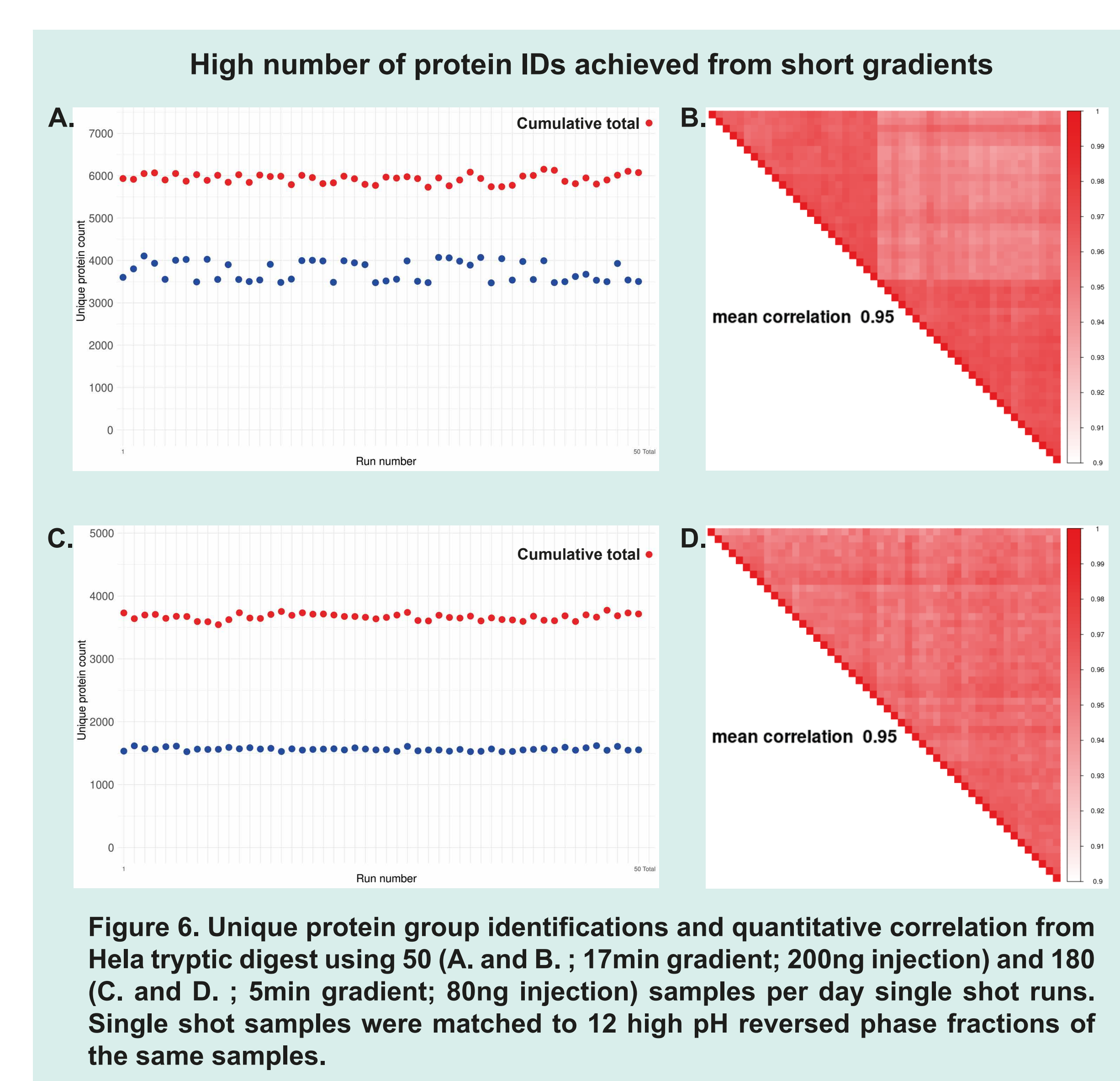
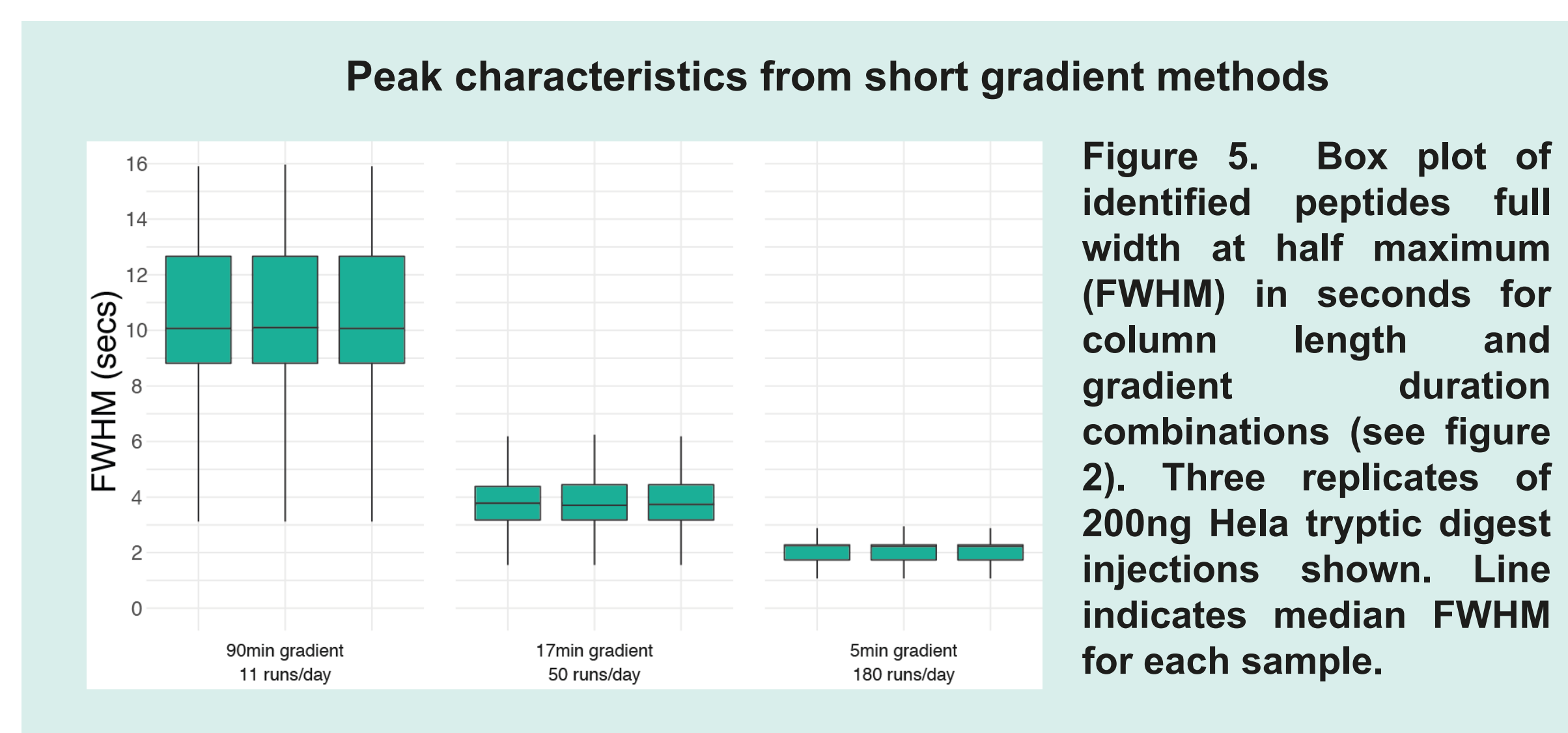
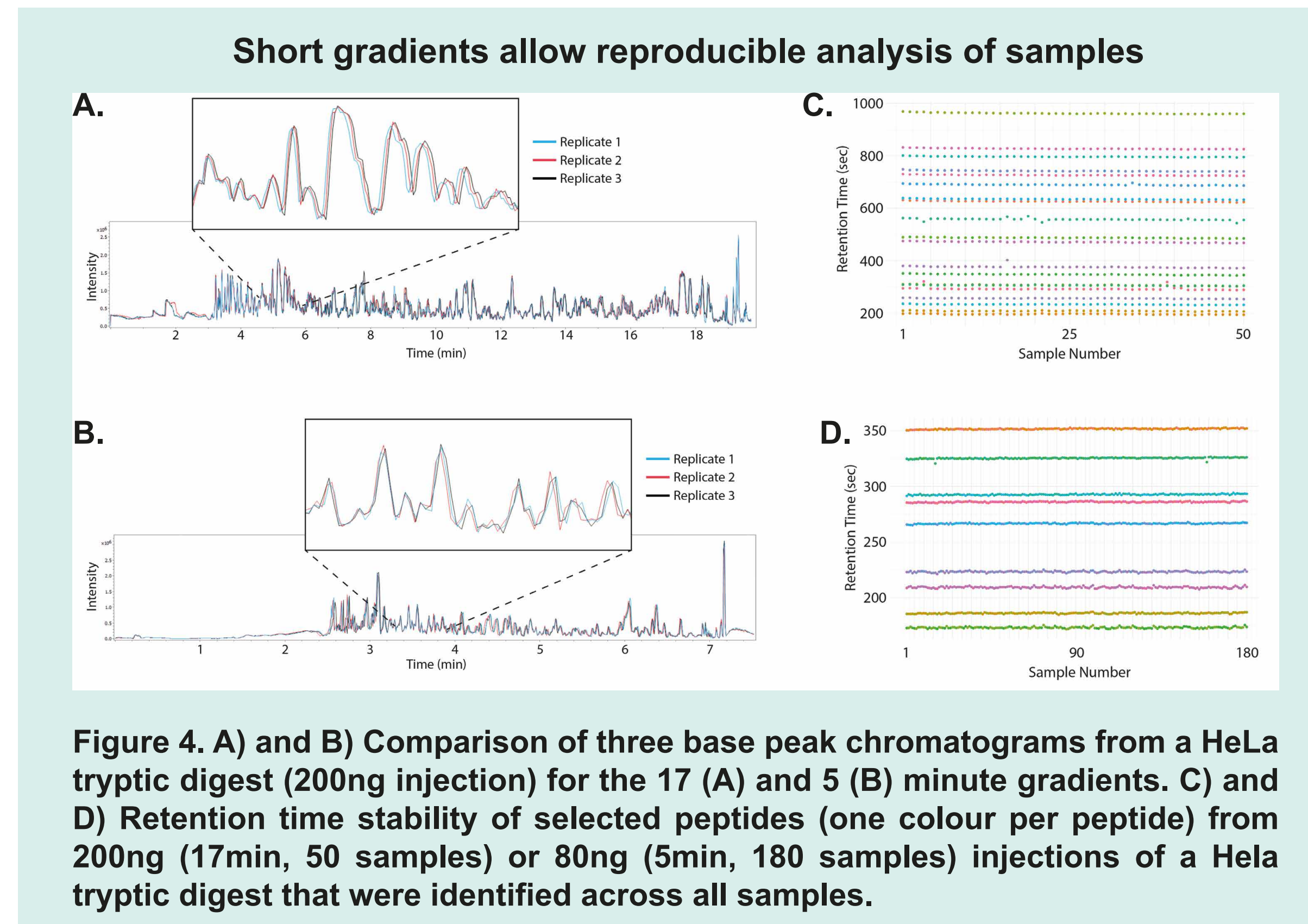
Established proteomic methodologies have relied on long analytical columns and extended UHPLC gradients to achieve comprehensive proteome coverage. The timsTOF Pro pushes effective fragmentation rates beyond 100Hz and provides accurate collisional cross section (CCS) where established methodologies underutilize 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 dimensional (4D) peptide data was acquired using data-dependent PASEF before analysis using MaxQuant (Figure 1).



## Results



## 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