#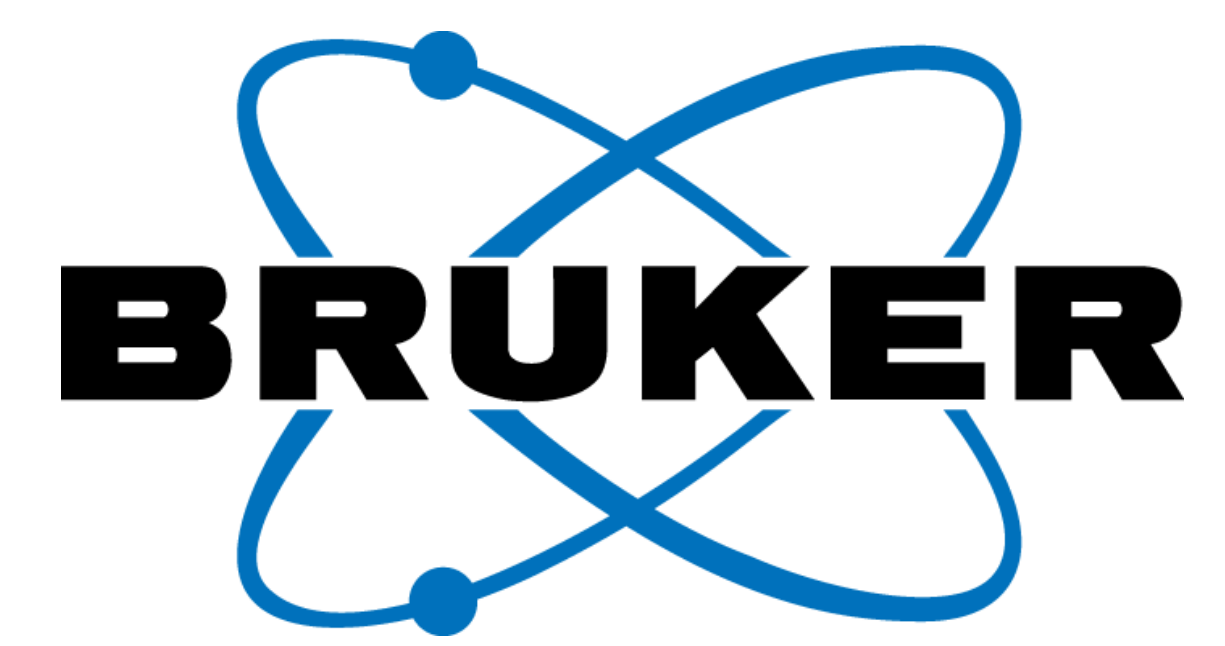 Maximized throughput and analytical depth for shotgun proteomics using PASEF on a TIMS equipped QTOF and a novel LC system

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Introduction

Mass spectrometry (MS)-based proteomics has become a powerful technology for the identification and quantification of thousands of proteins. While good analytical depth is currently achievable, the throughput required in screening or clinical applications is not achievable with traditional nano-ESI
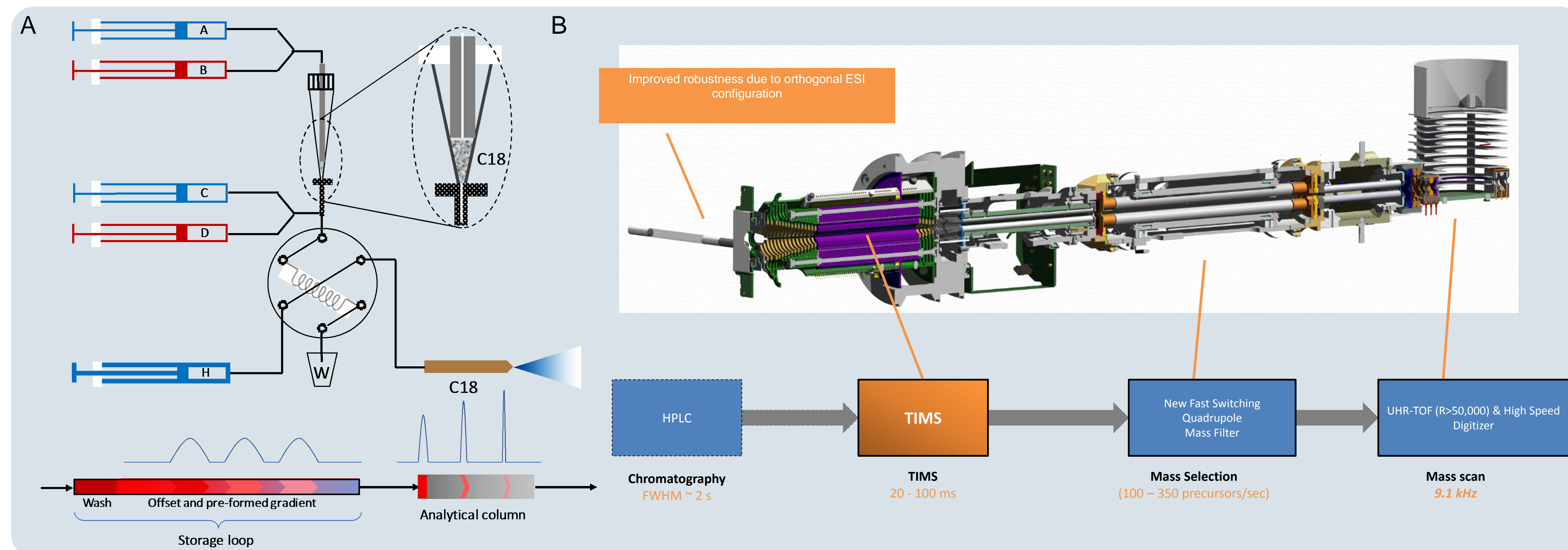

Fig. 1: Evosep One and timsTOF Pro. A) Illustration of the Evosep One separation principle. Peptides are eluted from Evotips with low pressure pumps (A/B) by increasing concentration of eluent B (ACN). Peptides and organic solvent are then diluted with low pressure pumps (C/D) and transferred on a storage loop. Pre-separated peptides are further separated on a short analytical column. B) TimsTOF Pro with trapped ion mobility spectrometer (TIMS) to increase peak capacity. Fast quadrupole switching allows selection of 100 – 350 precursors/sec in the PASEF mode to deal with the high complexity on short gradients.
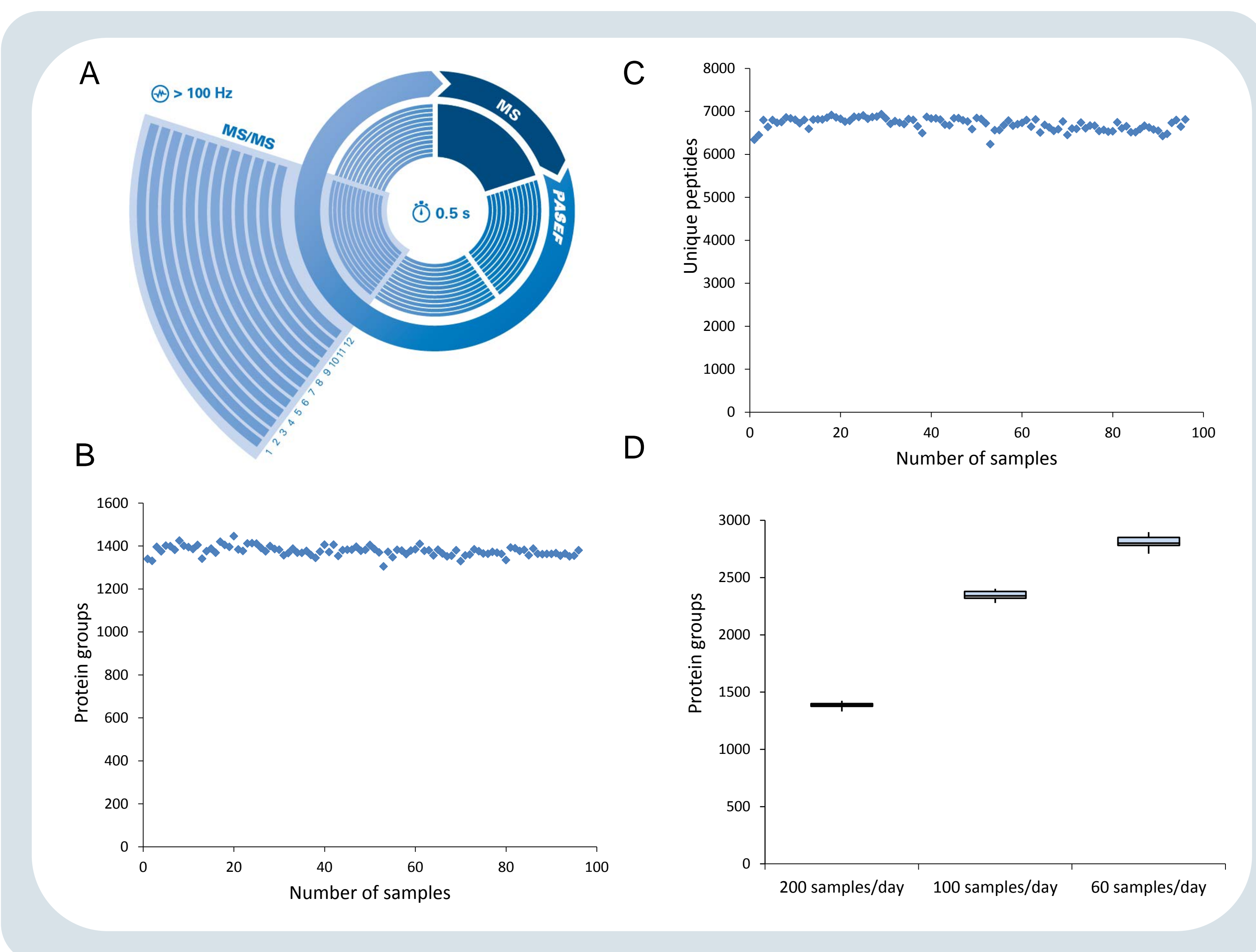

Fig. 2: Analytical depth and reproducibility. A) PASEF acquisition scheme for acquisition speed >100 Hz. B) Close to 1400 protein groups and C) 7000 unique peptides were reproducibly identified on the 200 samples/day method. D) ID results for 200 samples/day, 100 samples/day and 60 samples/day.

Fig. 3: Reproducible protein quantification. A) Measurement of 96 samples of 50 ng HeLa digest. Proteins can be quantified very reproducibly with R^2 values of around 0.97 over all 96 runs. B) Reproducible Total Ion Chromatogram intensities over 96 runs with a CV of 2.3%. C) Reproducible quantification of 7 selected proteins in different abundances.

LC-MS/MS systems. Hence, we combine the Evosep One with low overhead times and the timsTOF Pro with the fast Parallel Accumulation Serial Fragmentation (PASEF) acquisition method (Meier et al., JPR 2015). We show highly reproducible identification and quantification with low sample amounts over ~100 runs, demonstrating the potential for high throughput applications.

Methods

A HeLa protein digest was diluted in 0.1% formic acid and 50 ng were loaded on Evotips. In the Evosep One, peptides were first pre-separated on the Evotips for subsequent separation on the analytical column (**Fig. 1A**). The timsTOF Pro, with a dual TIMS cell for almost no ion loss (100% duty cycle) and ion mobility separation (**Fig. 1B**) was used for acquisition. Data were acquired using PASEF with a total cycle time of 0.5 s (**Fig. 2A**). Data analysis was performed with PEAKS and MaxQuant.

Results

Protein and peptide identification is very reproducible on 5.6 min runs (200 samples/day) with an outstanding number of 1400 protein groups and 7000 unique peptides, respectively (**Fig. 2B and C**). 100 and 60 samples per day can be reproducibly measured to a depth of 2300 and 2800 protein groups, respectively (**Fig. 2D**). Protein quantification across 96 HeLa replicate runs illustrate high reproducibility with R^2 values of around 0.97 (Fig. 3A). Robust measurements are also reflected by reproducible TIC intensities (median CV=2.3, **Fig. 3B**). On single protein level LFQ quantification is possible over several orders of magnitude with low variation on LFQ values across 96 runs (**Fig. 3C**).
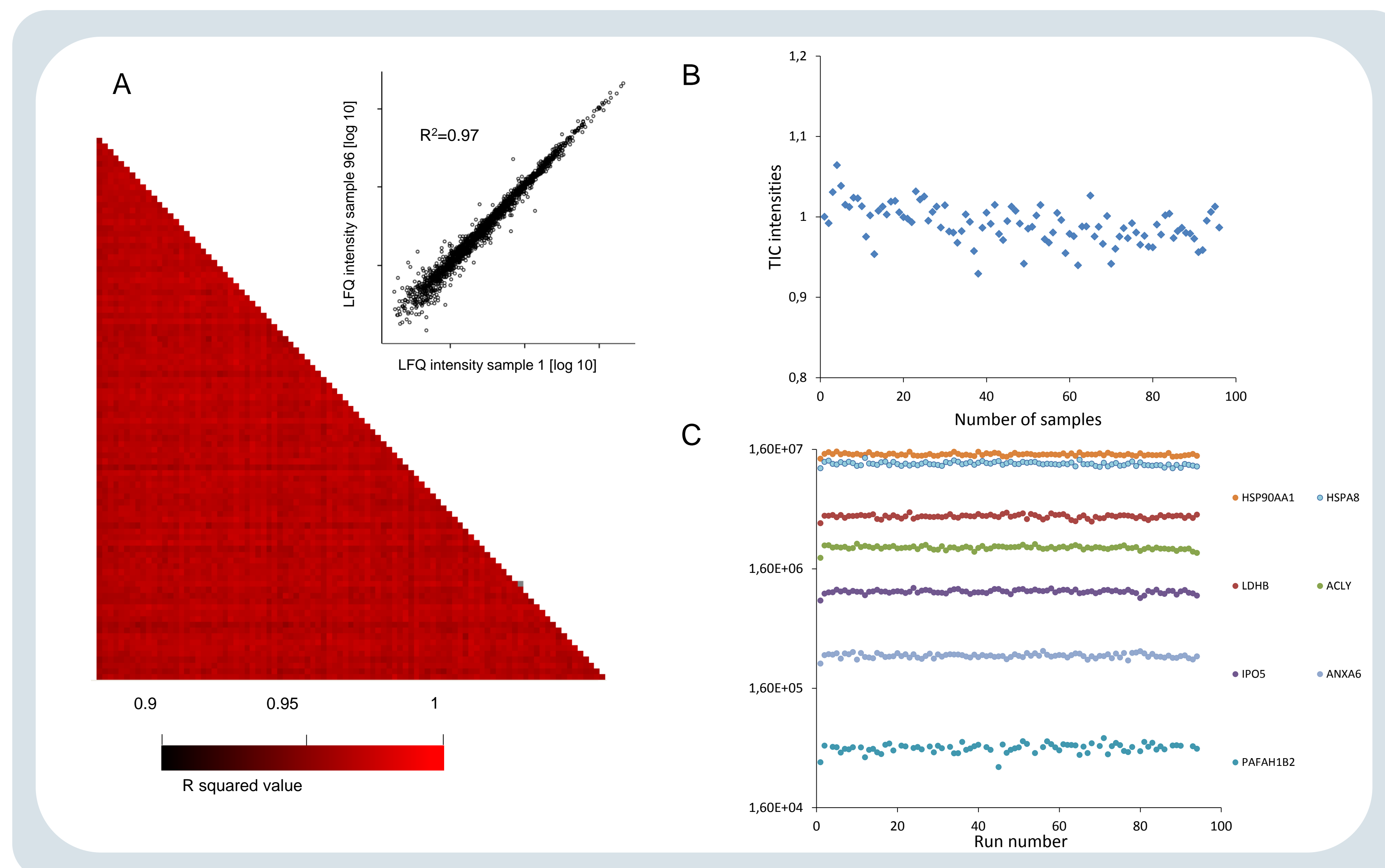
Conclusions

- **High sequencing speed** provided by PASEF for reasonable analytical depth even on very short runs

- **High reproducibility** of ID and quantification results demonstrating outstanding potential for high throughput studies of thousands of samples

- **High sensitivity** achieved by the 100% duty cycle of the timsTOF Pro, enables high throughput investigations with only 50 ng peptide digest/sample

timsTOF Pro