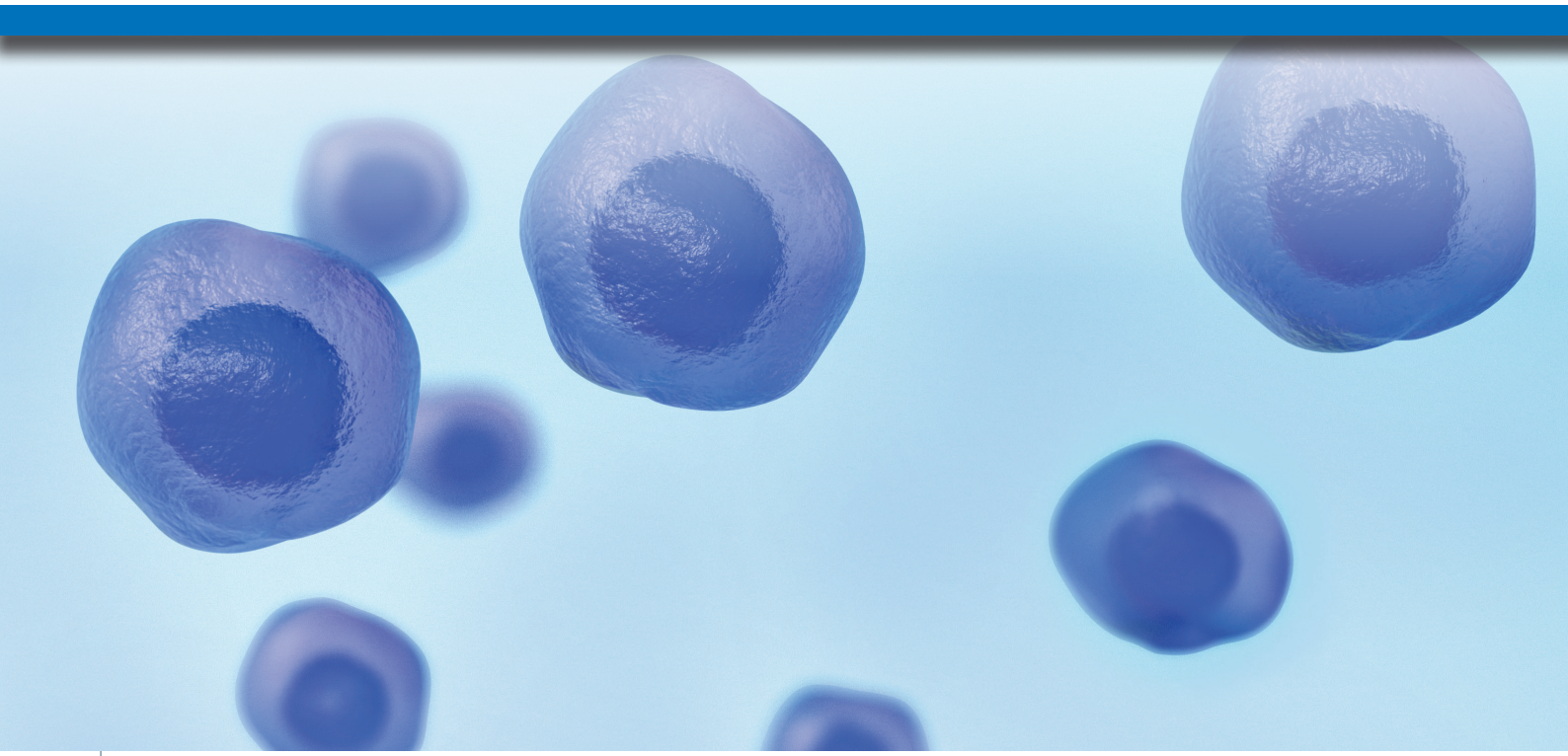

Accurate Label-Free Protein Quantitation on the timsTOF Pro with 4D-Proteomics™

The timsTOF Pro platform brings together two unique technologies, namely Trapped Ion Mobility Spectrometry (TIMS) mode operation at the front-end and Parallel Accumulation Serial Fragmentation (PASEF [1]) for data acquisition.

Abstract

This directly leads to two key benefits (a) mobility based time focusing of ions, boosting sensitivity, (b) efficient and intelligent usage of the ions for MS/MS analysis. Here we apply the PASEF™ strategy to perform label-free quantitative proteomics on a mixed-species sample. We demonstrate that PASEF on the timsTOF Pro yields high-precision quantitation for more than 8400 protein groups across three species within a reasonably short analysis time.

Introduction

Sample complexity, dynamic range of protein concentration and sensitivity of the mass spectrometer are the long-standing issues that preclude complete proteome analysis in a high-throughput fashion. This in turn has driven the technological advancement in acquisition speed and sensitivity of mass spectrometers. The timsTOF Pro mass spectrometer is a modified form of Q-TOF comprising of

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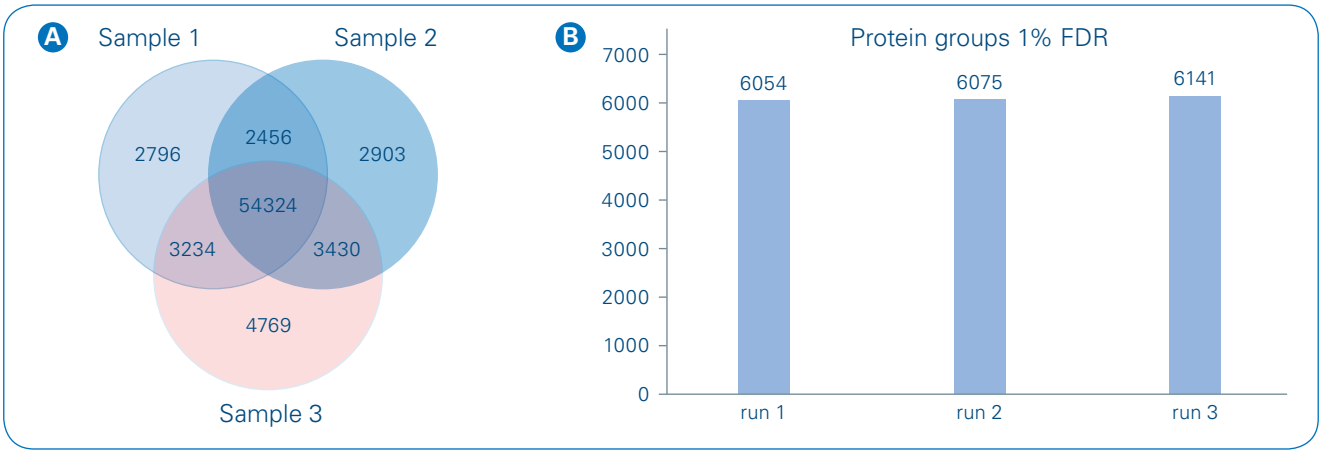

Figure 1: Number of identified peptides (A) and protein groups (B) from triplicate injections of HeLa cell digest.

trapped ion mobility spectrometry (TIMS) prior to a mass selecting quadrupole with operating time scales in the milliseconds range. The TIMS device thus bridges the gap between liquid chromatography and time-of flight scales while adding an additional dimension of separation. Time-focusing of accumulated ions within the TIMS device directly translates to about a 50-fold sensitivity gain enabling analysis of low sample amounts. With two TIMS sections operating in parallel within the funnel region we have developed the unique PASEF (Parallel Accumulation Serial Fragmentation) technology [1]. PASEF schedules multiple precursors for MS/MS analysis within a single TIMS-scan by synchronizing the mass selecting quadrupole with mobility elution profiles, making full use of the ions resulting in very high sequencing speed. Together these benefits in the timsTOF Pro mass spectrometer makes it a perfect fit for routine, rapid, in-depth and high sensitivity proteomics in any lab. Label-free quantitation is a widely used strategy for robust quantitative proteome profiling of a variety of samples. Here we demonstrate the powerful combination of the timsTOF Pro platform and label-free quantitation for analysis of tryptic digests of human, yeast and *E. coli* proteins mixed in pre-defined ratios.

Methods

Sample preparation

An in-house HeLa cell digest was used to qualify instrument performance. Whole HeLa cell pellets were purchased from CIL Biotech (Mons, Belgium). Cell lysis was performed using trifluoroethanol (TFE) as described elsewhere [2]. Briefly, the suspension was cooled on ice for 10 minutes and then incubated at 56°C for 20 min. Protein reduction was performed at 90°C for 20 min with 200 mM dithiothreitol (DTT) and then alkylated with 200 mM iodoacetamide (IAA) for 90 min at room temperature. Proteins were enzymatically cleaved overnight by adding trypsin in a 1:100 (wt/wt) enzyme:protein ratio. De-salting and purification was performed using a solid phase extraction cartridge (SepPak C18, Waters, USA) by diluting and washing protein digest with 0.1% formic acid (FA) and subsequent elution with 50% (w/w) acetonitrile (ACN) in 0.1% FA. Purified and dried peptides were reconstituted in 0.1% FA.

The samples containing tryptic digests of protein extracts from HeLa, yeast and *Escherichia coli* (*E. coli*) cells were prepared as described elsewhere [3]. To generate the hybrid proteome sample, tryptic peptides were combined in the following ratios: Sample HYE-A was composed of 65% w/w human, 30% w/w yeast, and 5% w/w *E. coli* peptides. Sample HYE-B was composed of 65% w/w human, 15% w/w yeast, and 20% w/w *E. coli* peptides. 400 ng of the mixed sample was loaded directly on the Bruker Daltonics nanoElute system (details see Table 1).

Table 1: nanoLC conditions

Parameter	Settings
LC system	nanoElute (Bruker Daltonics)
Separation Column	Reversed-phase C18 (25 cm x 75 µm i.d., 1.6 µm, IonOpticks, Australia)
Mobile Phases	A: 0.1% formic acid (FA) in water, B: 0.1% FA in Acetonitrile
Gradient	2 to 37% B in 100 min
Flow Rate	400 nL/min
Column Temperature	50°C

Mass Spectrometry Settings

Peptides were analyzed on the timsTOF Pro mass spectrometer using the unique PASEF technology (Table 1). The timsTOF Pro platform

is equipped with state-of-the-art dual-TIMS funnel ion optics that sorts and time-focuses ions before they enter the quadrupole-time-of-flight (Q-TOF) mass analyzer. PASEF synchronizes MS/MS precursor selection with TIMS separation. This allows scheduling more than one precursor per TIMS scan and increases the sequencing speed several-fold without loss of sensitivity. The precursor selection engine dynamically selects precursors based on intensity, *m/z*, and ion mobility.

Data processing

Data were processed using PEAKS Studio X+ (Bioinformatics Solutions Inc.). PEAKS Studio is a software platform with complete solutions for discovery proteomics, including protein identification and quantification. Database search parameters included: MS tolerance: 20 ppm, MS/MS tolerance: 0.05 Da, Oxidation of methionine and protein N-term acetylation were set as variable modifications and carbamidomethylation of cysteine as a fixed modification.

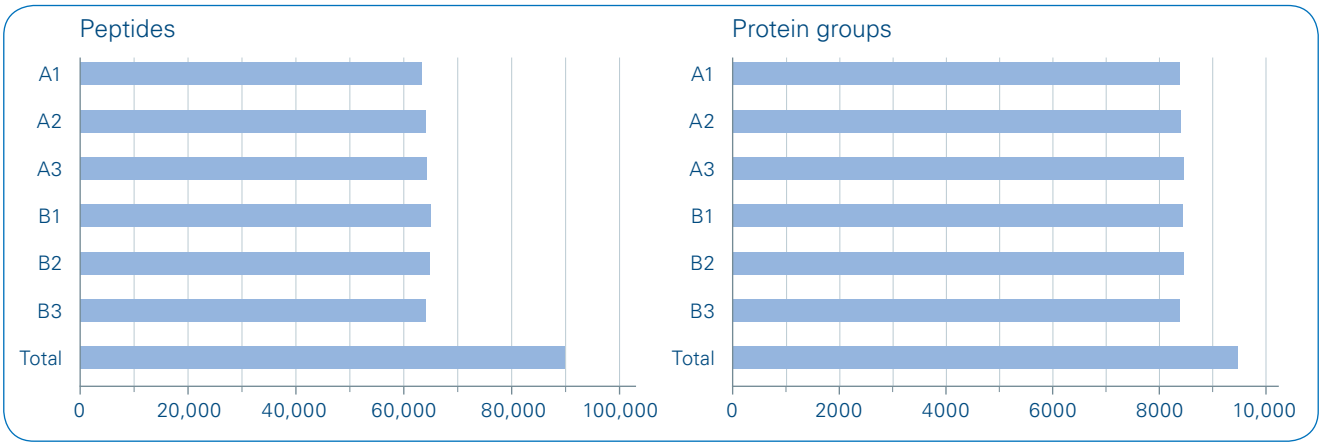

Figure 2: Number of identified peptides and protein groups per sample and total number of identifications.

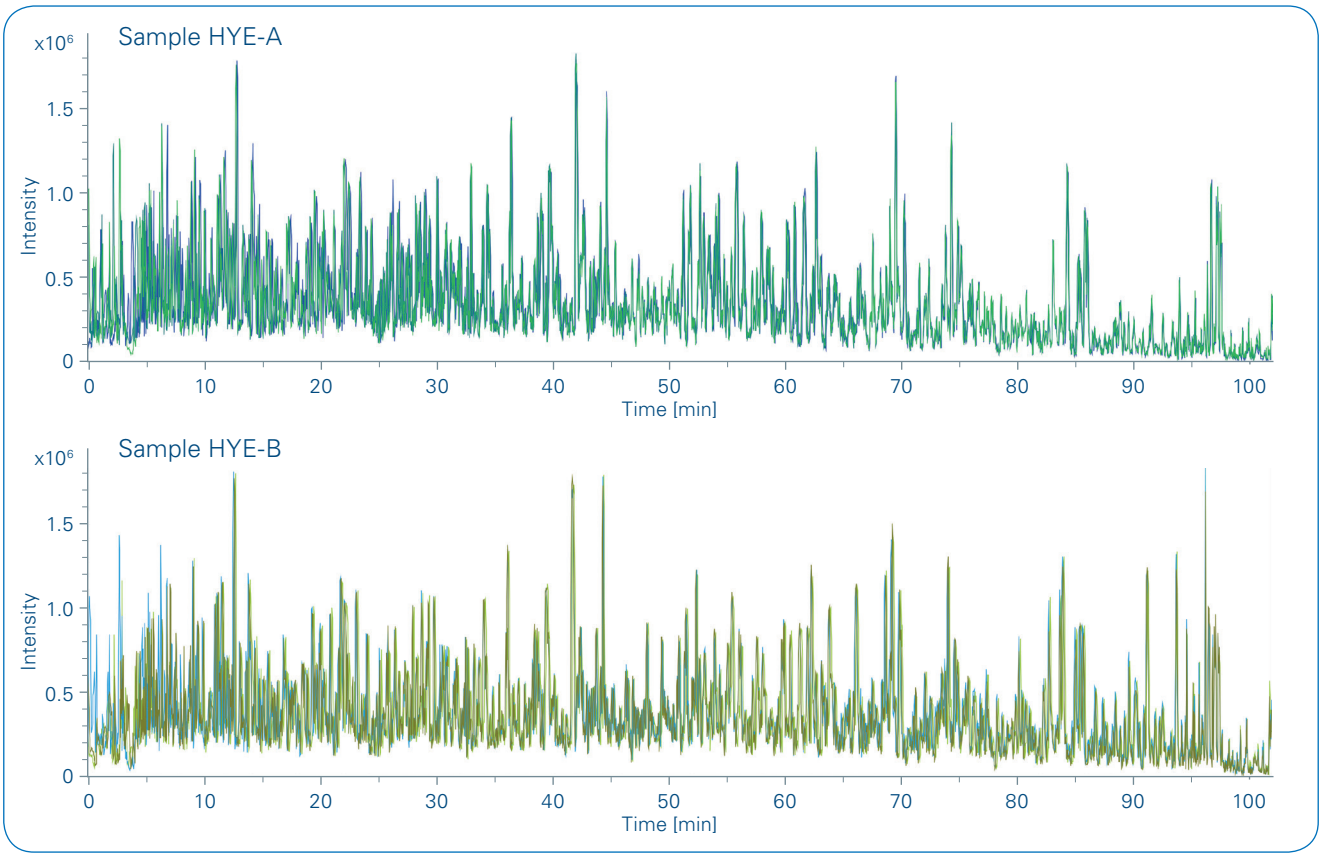

Figure 3: Overlay Base Peak Chromatogram MS for triplicate injections of the two proteomics samples, HYE-A and HYE-B, displaying the excellent reproducibility of the measurements regarding chromatographic separation and mass spectrometric detection.

Results and Discussion

We investigated the performance of the timsTOF Pro platform for the analysis of human cell lysate as well as for a complex three-proteome mixture consisting of HeLa, yeast and *E. coli*.

First we checked the overall performance of the instrument using an in-house HeLa cell digest. The number of identified protein groups and peptide sequences is typically used to evaluate LC-MS/MS performance. From triplicate injections separated with a 100-min gradient we could identify on average 63,893 peptides with 85% of them identified in all three runs. On the protein level ~6.1k protein groups could be identified in a single PASEF run (Figure 1).

In a next step, we investigated the performance of the PASEF technology using the timsTOF Pro for label-free quantitation of complex proteomic mixtures. To evaluate the quantitative capabilities, we compared two samples both consisting of tryptic digests of human, yeast and *E. coli* cell lysates. The three proteomes were mixed in defined ratios (1:1 for human, 2:1 for yeast an 1:4 for *E. coli*) with human proteins representing the background proteome. These samples provide defined relative changes between the samples together with a sufficiently large number of peptides to enable the in-depth evaluation of both precision and accuracy of the label-free approach.

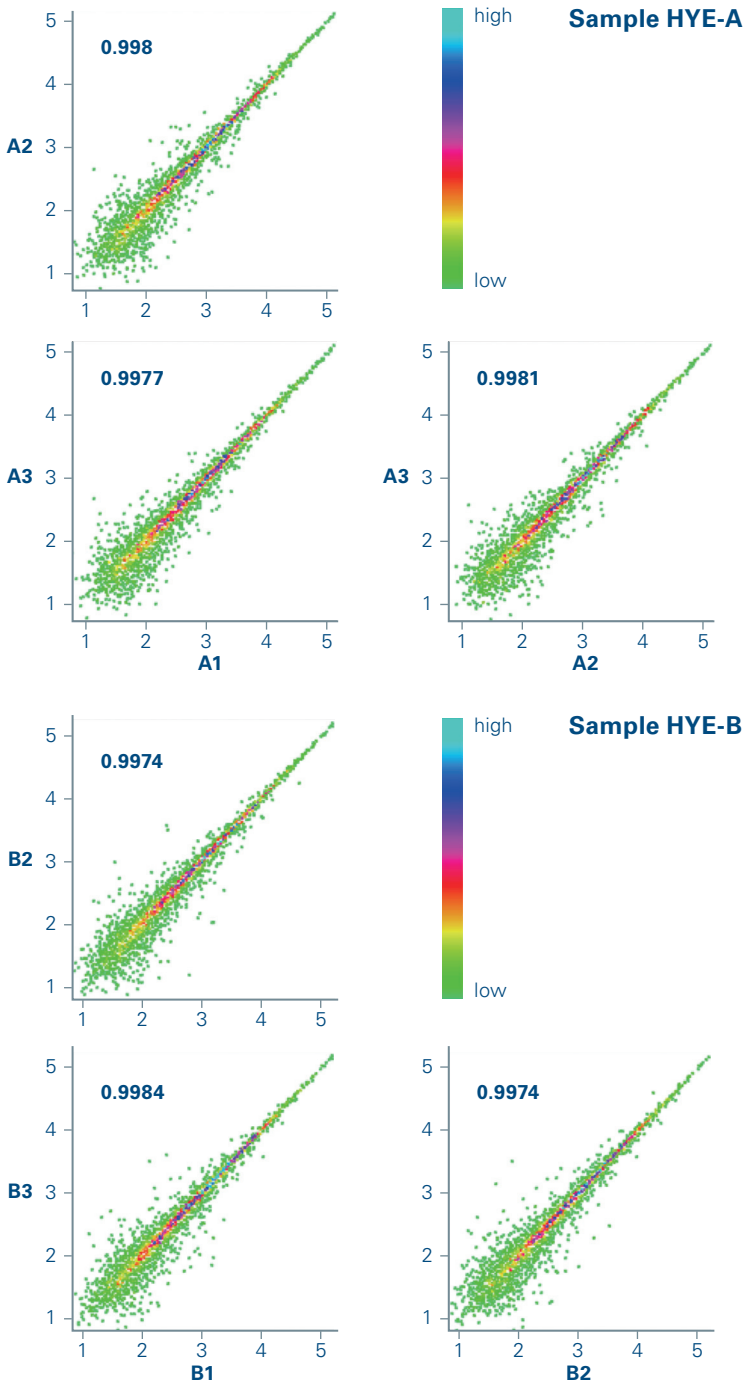

Figure 4: Sample correlation on protein group level for the two samples, HYE-A and HYE-B.

The samples were measured in triplicate. PEAKS X+ analysis using a combined human, yeast and *E. coli* database identified on average 64,334 peptide sequences and 8410 protein groups in the three-proteome mixture. This results in a total identification of 89,835 peptide sequences and 9467 protein groups for the six analyzed data sets with a 100-min gradient (Figure 2) using the "ID transfer" between runs algorithm in PEAKS X+.

Reproducibility and accuracy of quantitation determines the confidence of any proteomics methodology. In the present study, LC-MS separation between replicates was very reproducible as can been seen in Figure 3.

This excellent chromatographic reproducibility in combination with accurate mass spectrometric detection at the MS1 level resulted in very high reproducibility of protein and

peptide quantitation. Average correlation values of 0.99 are observed between the technical replicates of sample HYE-A and sample HYE-B (Figure 4) demonstrating the excellent reproducibility of MS1 – level label-free quantitation of the timsTOF Pro platform.

The high data completeness and reproducibility in this study is also a result of the "ID-transfer" between runs algorithm in PEAKS X+, which uses a 4-dimensional matching algorithm including matching of CCS-values, significantly reducing the number of missing values.

The experimental design enabled the evaluation of the accuracy of label-free quantitation using the PASEF approach in a complex proteomics mixture with known ratios. Background human proteins were spiked in equal amounts resulting in a theoretical ratio of 1:1. With the quantitative precision demonstrated, the human population clustered nicely around log10 ratio of sample HYE-A versus HYE-B of 0 across the complete dynamic range (Figure 5). For yeast and *E. coli* proteins excellent global accuracy was achieved with determined ratios close to the theoretical ones (median ratios sample HYE-A/HYE-B for yeast: 2.5 (expected value: 2.0) and for *E. coli*: 0.2 (expected value: 0.25)).

Table 2: Mass spectrometry conditions

Parameter	Settings
MS System	timsTOF Pro platform (Bruker Daltonics)
Acquisition Mode	PASEF
MS *m/z* range	100 to 1,700
Number of PASEF MS/MS scans per cycle	10
Average number of precursors selected for each PASEF MS/MS scan	12
TIMS Ramp Time	166 ms
MS/MS Collision Energy settings	20 to 59 eV

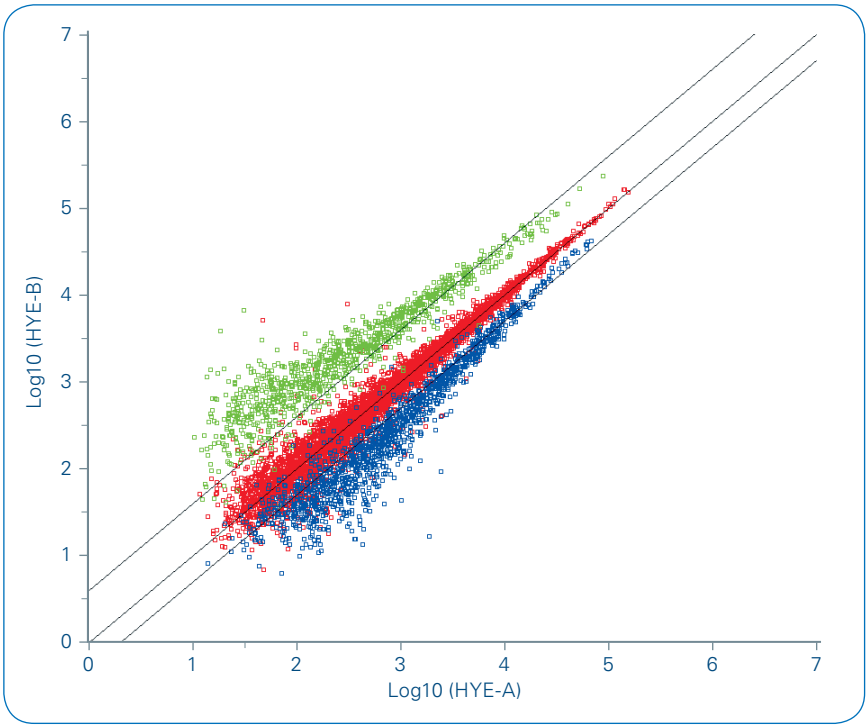

Figure 5: Protein level quantitation results. Log-transformed (log10) intensities of the average values of sample HYE-B and sample HYE-A were plotted against each other (red = human, blue = yeast, green = E.coli*). Lines represent the expected values.*

Conclusion

- More than 8400 proteins could be reproducibly quantified from a hybrid proteome mixture in single 100-min LC runs taking advantage of the unique capabilities of the timsTOF Pro platform including high ion usage efficiency from the TIMS device and the "ID transfer" feature in PEAKS X+ software.
- The timsTOF Pro with PASEF technology delivers reproducible identification and quantitation information over a concentration range of 5 orders of magnitude making it perfectly suited for label-free quantitation.

Learn More

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Check out the link or scan the QR code for more details.

www.bruker.com/timstofpro

References

[1] Meier F et al (2018). Mol Cell proteomics, doi: 10.1074/mcp.TIR118.000900

[2] Wang et al. (2005). J Proteome Res., **4**(6): 2397-2403

[3] Navarro P et al. (2016). Nat Biotechnology, doi: 10.1038/nbt.3685

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