Integrating Advanced Ion Activation with High Sensitivity on timsOmni: A Versatile Platform for Biological Discovery

Stephanie Kaspar-Schoenefeld[1], Andreas Schmidt[1], Daniel Hornburg[2], Dimitris Papanastasiou[1], Markus Lubeck[1]

[1]Bruker Daltonics GmbH & Co. KG, Bremen, Germany, [2]Bruker Daltonics Inc, Billerica Massachusetts, United States

Introduction

Understanding the complexity of biological systems at the molecular level requires tools that can sensitively and accurately characterize proteins across a wide dynamic range. The recently introduced timsOmni instrument, equipped with a unique ion processing system that provides a variety of ion activation-dissociation techniques (MSn, eXd), offering unique capabilities for top-down proteomics enabling detailed structural and functional analysis of intact proteins and proteoforms (Fig. 1). Despite its specialization, the timsOmni is built on the timsUltra AIP platform, which is designed for bottom-up proteomics, providing the particularly high sensitivity required for single-cell and other sample-limited applications. In this study, we evaluate the shotgun proteomics capabilities of the timsOmni, with the omnitrap component operating in pass-through mode.

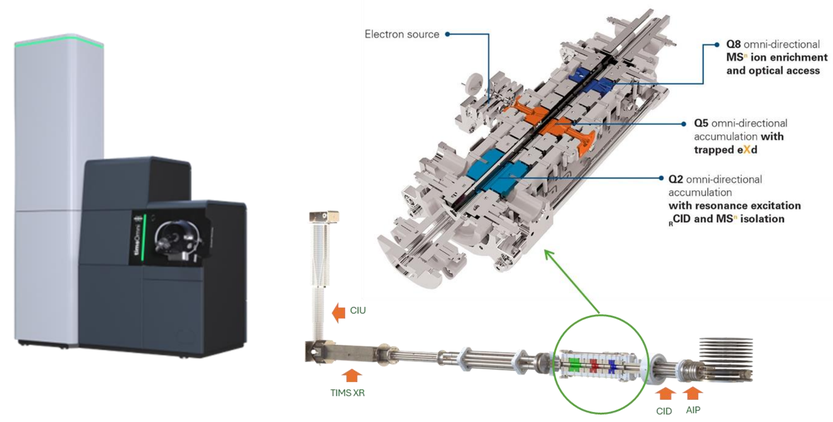

Figure 1: Overview of the timsOmni instrument. timsOmni features unparalleled full scan MS and MS^n sensitivity with access to the entire eletron fragmentation landscape.

Material & Methods

To evaluate the proteomics performance of the timsOmni instrument, tryptic digests of the human K562 cell line (Promega) were diluted in 0.1% formic acid containing 0.015% n-Dodecyl-β-D-Maltoside (DDM). A triple proteome mixture was prepared in-house by combining individual digests from HeLa cells, Yeast, and E. coli. Samples were separated using a ProteoElute nanoHPLC system (Bruker) equipped with PepSep Advanced columns (75 µm ID, 25 cm length) maintained at 50°C. The system was coupled to a CaptiveSpray Ultra 2 source with a 10 µm ID emitter. A linear gradient from 5% to 35% acetonitrile in 0.1% formic acid over 22 minutes at a flow rate of 250 nL/min was applied for all sample types. Data were acquired in dia-PASEF mode using a 166ms TIMS separation spanning 0.64–1.45 Vs/cm².

The dia-PASEF method employed five isolation windows, with the quadrupole isolation position stepped twice per TIMS scan. The data files were processed in Spectronaut (v20.2).

Results

Here we evaluated the proteomics performance of the timsOmni mass spectrometer operating the omnitrap component in pass-through mode. First, we focused on analyzing whole cell K562 lysates from single cell level (250pg) to 20ng. By applying a py-diAID optimized dia-PASEF method (Fig. 2A) we observed more than 5000 protein groups from single cell level injection amounts (on average 5082 from 250pg; increases to on average 5858 when matching IDs across the complete dilution series) and 7470 protein groups from 88k peptides for 20ng injection amounts (Fig. 2B).

In a next step we prepared a controlled quantitative experiment (CQE) by combining three different species (human, yeast and E. coli) in defined different ratios (Fig. 3A). From 25ng injection amounts, we identified more than 11650 protein groups (92k peptides) from both samples sets from quadruplicate injections (Fig. 3B), with 75% of those proteins being detected with CVs < 10%. The experimental setup yields in predefined fold changes when measured together. The amount of human peptides was kept constant, resulting in detected protein quantities being centered at a log-value of 0. The yeast content was at 25% at sample 1 and 15% for sample 2 and E.coli fraction increased from 15% to 25%, thus resulting in comparable regulation ratios however in different directions. Of the identified proteins, 3091 matched to yeast and 2034 to E.coli. Yeast protein group abundance ratios were observed at 1.5 and E.coli at 0.7 (Fig. 3C), both being close to the expected ratios (1.67 and 0.6, respectively) confirming the ability of the timsOmni to exactly differentiate changes in abundance making it perfectly suited for investigation of proteome-wide changes in protein abundance in different biological contexts.

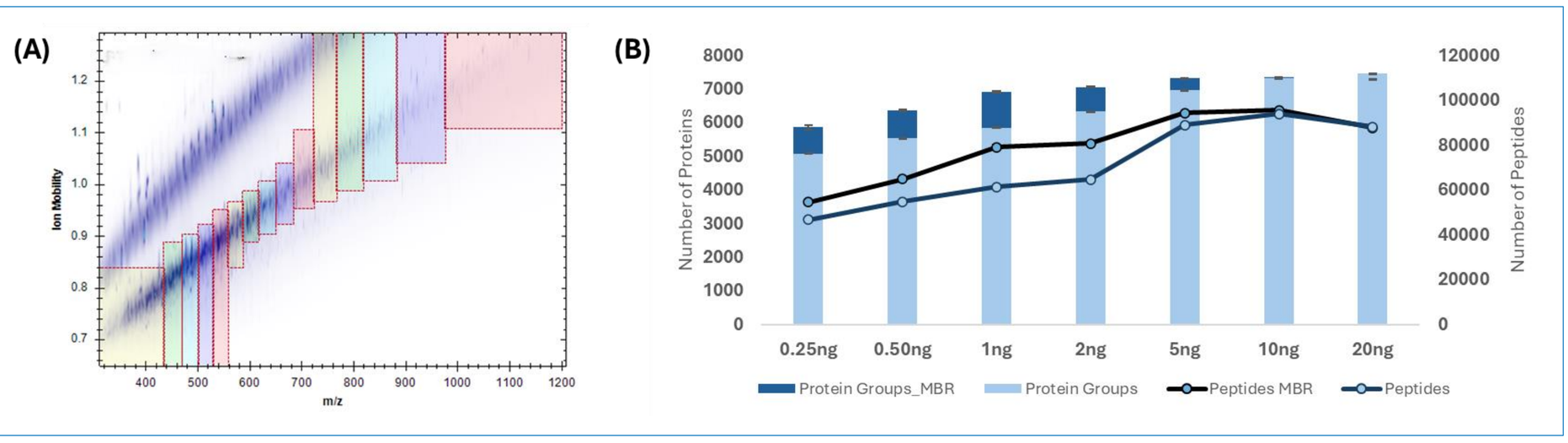

Figure 2: Proteomics performance of the timsOmni. (A) Applied dia-PASEF method generated via py-diAID. (B) In-depth protein identification using library-free Spectronaut data processing with and without matching for a dilution series ranging from 250pg K562 to 20ng on a 22 min gradient.

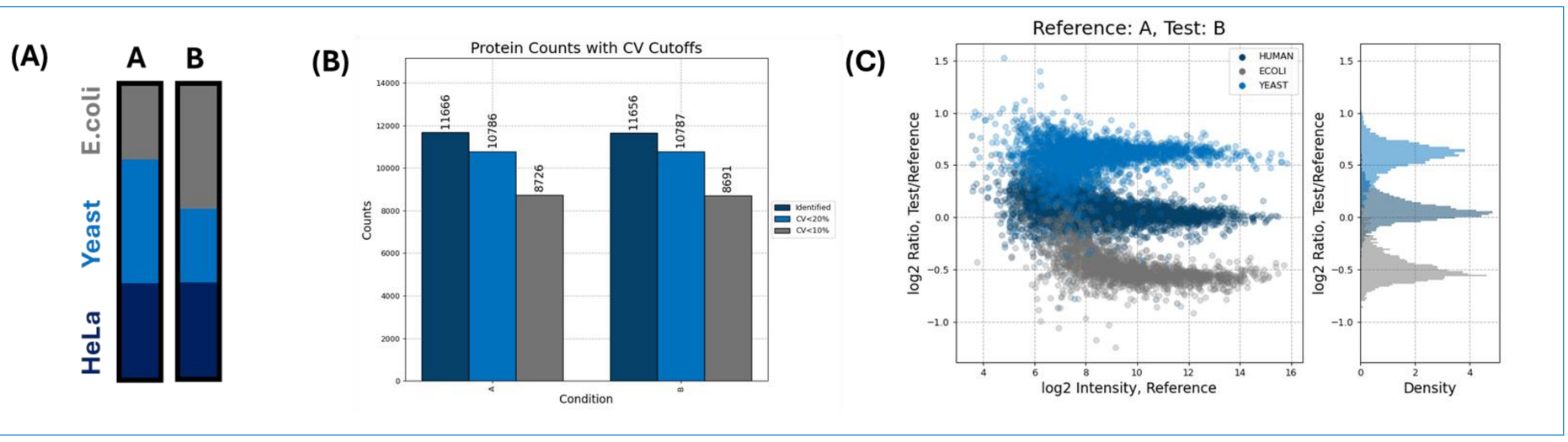

Figure 3: Determination of the quantitation performance of the timsOmni. (A) Sample composition for hybrid proteome analysis. (B) Number of total protein groups identified and quantified using library-free Spectronaut data processing as well as numbers below 10% and 20% of coefficient of variation (CV) from quadruplicate injections. (C) Measured log2 abundance ratios for each of the organism spiked-in.

- The timsOmni capable of MSn and eXd retains highest sensitivity and quantitative accuracy for analysis of complex proteomics samples making it a powerful tool for systems biology, biomarker discovery, and functional proteomics.

ProteoElute+ timsOmni

Conflict of interest: SK-S, AS, DH, DP and ML are employees of Bruker Daltonics.