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The timsTOF Ultra enables deep global ubiquitinomics of ultra-low protein input samples for validating degrader drug targets

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Introduction

Targeted protein degradation (TPD) is a promising new drug modality to remove specific cellular proteins. The assessment of degraders for TPD via mass spectrometry (MS)-based proteomics is particularly appealing for the unbiased discovery of novel targets for molecular glues, since rational drug design proves challenging for those molecules. Once identified by global proteomics, such drug target candidates need to be mechanistically validated, for example by demonstrating drug-induced target protein ubiquitination. We present a global ubiquitinomics workflow for detecting degrader drug induced protein ubiquitinations of ultra-low protein input samples, by combining an improved ubiquitin-remnant peptide enrichment workflow with single-shot LC-MS on the timsTOF Ultra, and a custom-made statistical analysis pipeline.

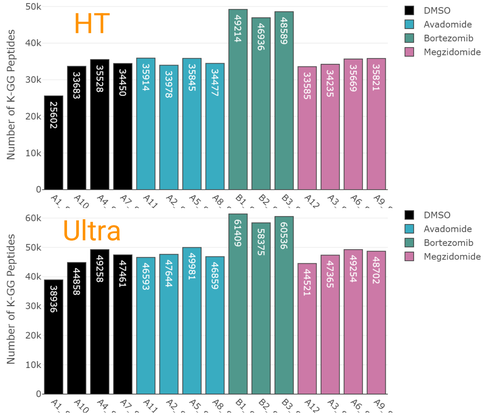

Fig. 1: Number of K-GG Peptides identified after processing raw data in dia-NN using a predicted library and subsequent statistical analysis using an in-house developed pipeline. Upper plot shows timsTOF HT data, while the lower plot shows timsTOF Ultra data.

Methods

All samples were analyzed using a nanoElute® 2 (Bruker Daltonics) equipped with an Aurora Ultimate CSI 25 cm column (IonOpticks) coupled to either a timsTOF HT (Bruker Daltonics) or timsTOF Ultra (Bruker Daltonics). Chromatographic separation was done at 50°C on a 38 min active gradient with 45 min total runtime and a flow rate of 250 nL/min. The mass spectrometers were operated in dia-PASEF® mode using a slice-PASEF (1 frame - 15 windows) scheme. Raw data processing was performed using dia-NN 1.8.2 beta 27 with QuantUMS and an in-house developed pipeline was used for downstream statistical analysis.

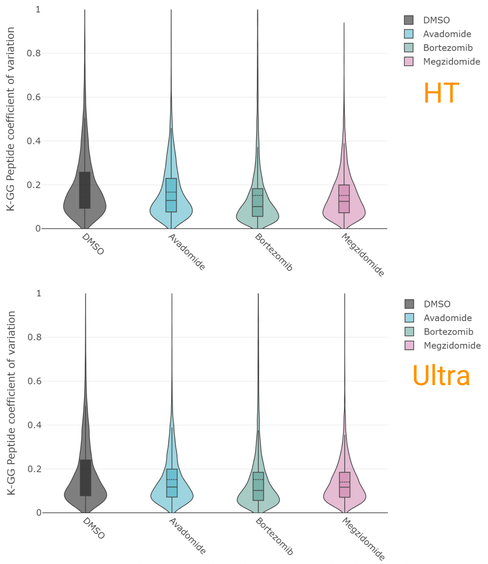

Fig. 2: Coefficient of variation for of K-GG peptides by condition. Upper plot: timsTOF HT. Lower plot: timsTOF Ultra.

Results

Low protein input samples (100 µg, HEK293) were either treated with DMSO, Avadomide or Megzidomide in four replicates for 30 min and enriched for ubiquitin-remnant peptides (diGly of K-GG peptides), subsequently. Additionally, a triplicate of higher protein input (300 µg) was treated with proteosome inhibitor Bortezomib to boost identifications. Samples were split in three and analyzed on timsTOF HT and timsTOF Ultra and processed in dia-NN using a predicted spectral library.

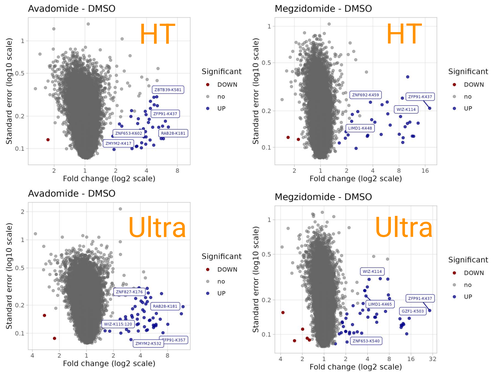

Fig. 3: Volcano plots of quantified K-GG peptides showing Avadomide or Megzidomide compared to DMSO. Upper plot: timsTOF HT. Lower plot: timsTOF Ultra.

Identified K-GG peptides ranged between 38936 and 49981 (Fig. 1), which represents a gain of between 24% and 52% when comparing to the timsTOF HT results, demonstrating the benefit of the high sensitivity of the timsTOF Ultra. The highly efficient enrichment was shown by the ratio of K-GG precusors to all precursors, which were on average 91% for timsTOF HT and 94% for timsTOF Ultra measurements. Utilizing the high scanning speed (FWHW in scans: 4.5) of the timsTOF instruments lead to an excellent quantitative precision (Fig. 2).

The number of regulated features quantified on the timsTOF Ultra setup were 46 for Avadomide and 53 for Megazidome, which corresponds to 12% and 56% increase, respectively. The volcano plots for the two compounds showing results from timsTOF HT and Ultra measurements can be found in Fig. 3.

The ubiquitinomics data was also compared against data using a proteomics approach with 5 h treatment and no enrichment. Using t-statistic comparison, it can be shown that the ubiquitinomics data is in good agreement with the proteomics data (Fig. 4) for both timsTOF HT and Ultra measurements.

Summary

The herein presented data shows that both timsTOF HT and timsTOF Ultra are capable of detecting drug-induced ubiquitination events with excellent quantitative precision. The high sensitivity of the timsTOF Ultra proved to be highly valuable for the analysis of low protein input ubiquitinomics samples.

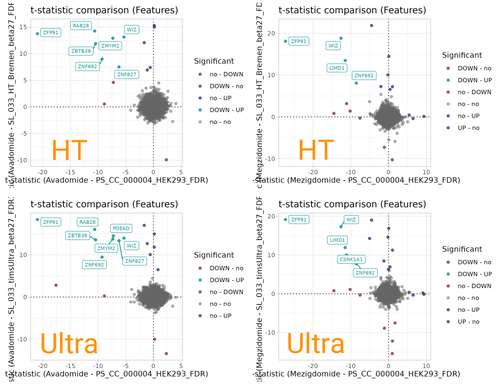

Fig. 4: t-Statistical comparison of candidates in ubiquitinome and proteome. Upper plots show results from timsTOF HT and lower plots from timsTOF Ultra measurements.

- Low protein input samples were treated with degrader drugs and subsequently measurement on timsTOF HT and timsTOF Ultra
- K-GG peptides were quantified with excellent quantitative precision
- The high sensitivity of the timsTOF Ultra proved to be highly valuable to identify degrader drug-induced ubiquitination events

Technology

PS, TM, and CA are current employees of Bruker Daltonics

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