Label-free quantitation of proteins and peptides using ZT Scan DIA

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ABSTRACT

The goal of proteomics is the quantitative characterization of proteins in biological systems and the correlation of changes in protein abundance to mechanisms of disease. Mass spectrometry-based proteomics is capable of characterizing proteins not only qualitatively but quantitatively as well. Data-independent analysis (DIA) is a commonly-used technique towards this end (1,2). Instrumentation and workflow innovations are needed to enable easier, faster, deeper and more sensitive quantitative identifications of proteins and peptides. ZT Scan DIA is a novel technique that employs a continuously scanning quadrupole to isolate precursor ions for fast, sensitive, time-of-flight (TOF) detection (3,4). In this work, ZT Scan DIA system was used for the label-free quantitation (LFQ) of complex lysate digests mixed at different ratios and analyzed at different on-column loadings. Fast microflow chromatographic separation was employed, using varying active gradient lengths. The resulting data was analyzed using DIANN software. The results show improvements in the precision and accuracy for LFQ using ZT Scan DIA compared to traditional discrete-window DIA methods. Collectively, these results highlight the effectiveness of ZT Scan DIA (using easy-to-set-up methods) for highly accurate quantitation of proteins, providing higher confidence to researchers for disease biomarker discovery.

MATERIALS AND METHODS

Sample preparation

Human K562 and yeast protein tryptic digest were purchased from Promega. E. coli tryptic digest was purchased from Waters. Samples were reconstituted in water with 0.1% formic acid and mixed at different nanogram ratios to prepare the seven mixtures as shown in Figure 1.

Chromatography

LC separations were performed with a Waters M-Class UPLC system in direct-inject LC mode. Microflow separations were performed using the indicated gradient lengths using a Phenomenex XB-C18 analytical column (0.3 mm x 150 mm), at a flow rate of 5 µL/min. Nanoflow separations were done using an IonOpticks Aurora SX Frontier (60 cm x 75 µm). The total on-column loading amount used is indicated for each experiment. Mobile phases consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). Each species digest was also subjected to high pH fractionation (5 fractions per species) followed by reverse-phase nanoflow separation and Zeno SWATH DIA analysis to build a spectral library for subsequent data processing (Figure 2).

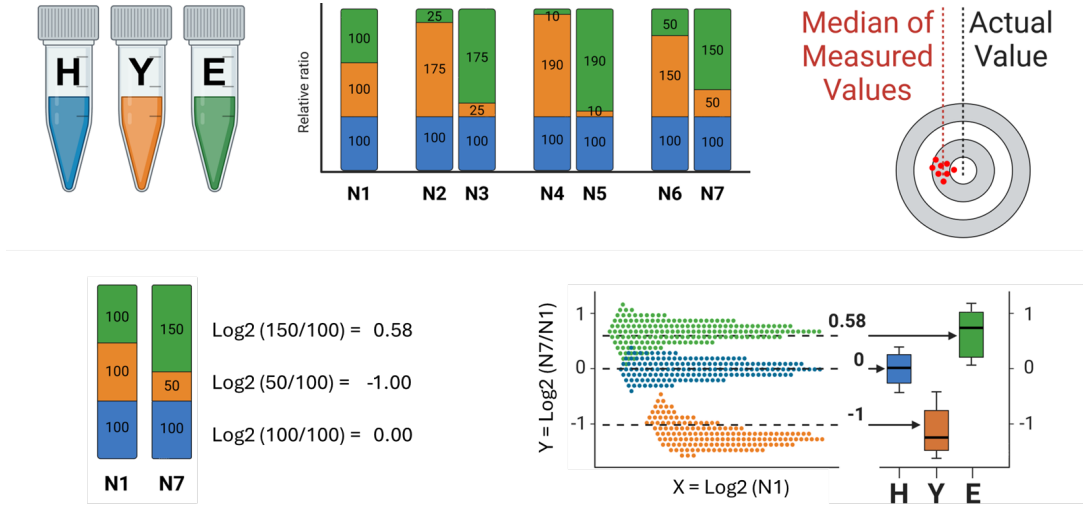


Figure 1. Overview of the label-free quantitation (LFQ) experiments. Seven different mixtures of human (H), yeast (Y) and E. coli (E) lysate tryptic digests were prepared (N1-N7). LC-MS injections of these samples were run on the ZenoTOF 7600+ system using either traditional discrete-window DIA (Zeno SWATH DIA) or ZT Scan DIA. After data processing using DIANN software, the ratios of protein groups and precursors for each species were determined for each mixture N2-N7 relative to the reference sample (N1), expressed as Log2 ratios. The deviation in the median of measured values relative to the theoretical values was determined for each organism in the mixture to determine the quantitation accuracies for each species.

MATERIALS AND METHODS

Mass spectrometry

Data-independent acquisition experiments were performed on a ZenoTOF 7600+ system. Zeno SWATH DIA experiments used 65 variable-width windows spanning the TOF MS mass range 400-900 Da, MS/MS mass range 140-1750 Da, with MS/MS accumulation (i.e. dwell) times as indicated, and Zeno trap pulsing turned on. ZT Scan DIA experiments used an estimated peak width at half-height (PWHH) setting of ≤ 1 sec (i.e. 7 msec precursor dwell times) or ≤ 4.5 sec (13 msec precursor dwell times). Data was acquired in triplicate for all conditions.

Data processing

All data was processed using DIA-NN software version 1.9.2 (5). For ZT Scan DIA data processing, the --scanning-swath command option was used. Searches were done for triplicate data files for a given experimental condition against the spectral library generated from the high pH fractionation of species' digests. Protein group and precursor numbers were obtained from the resulting output pg_matrix.tsv and pr_matrix.tsv files.

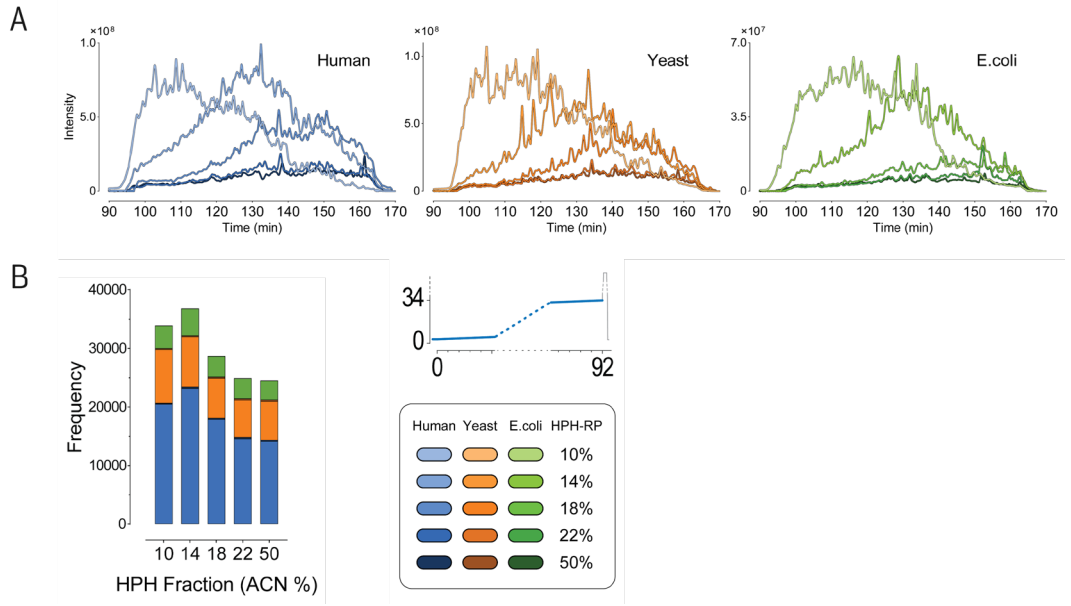
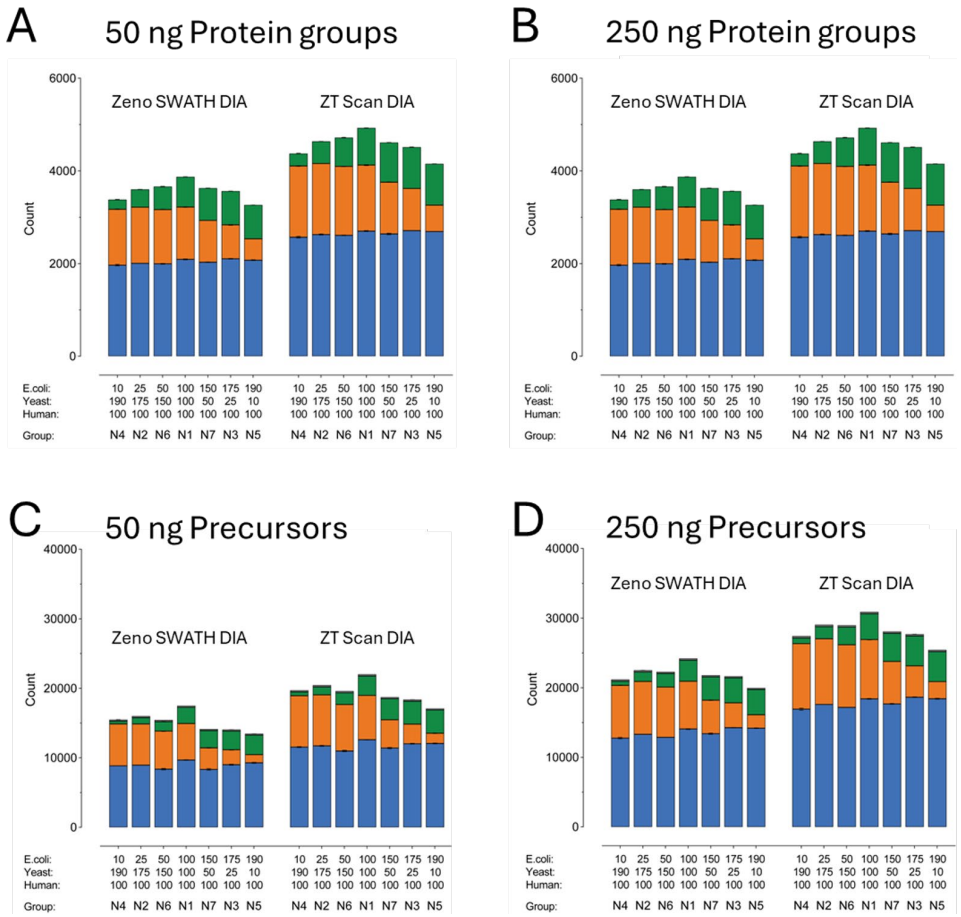


Figure 2. High pH fractionation of each species to build a spectral library. Each species was fractionated into 5 fractions using high pH fractionation (A) followed by 92-minute nanoflow reverse-phase LC-MS analysis using Zeno SWATH DIA. Figure 2B shows the frequency of peptides for each species in each of the 5 fractions.

RESULTS



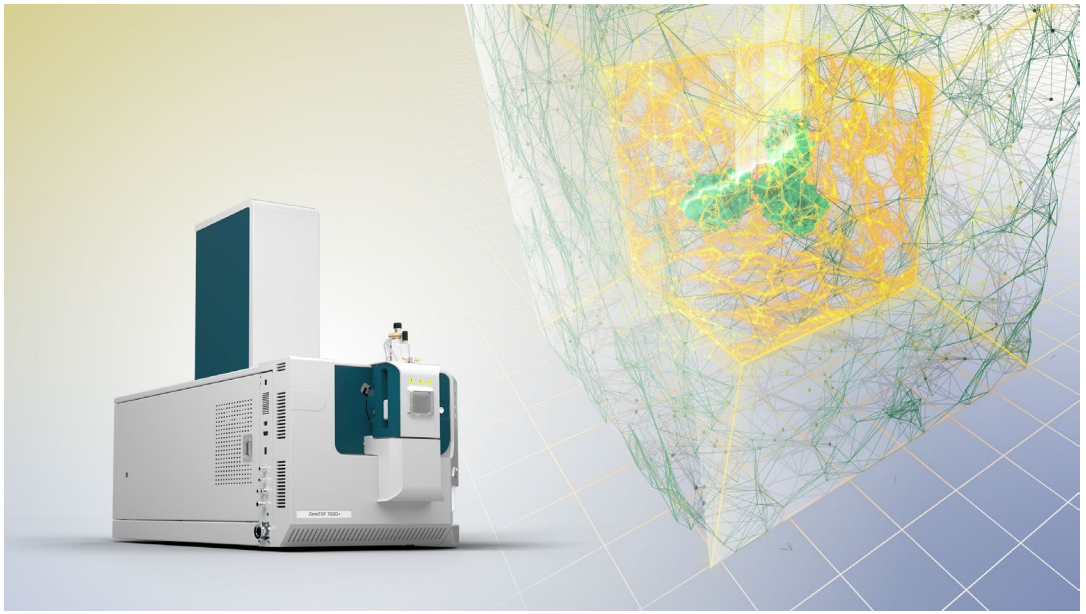


Figure 3. Protein groups and precursors identifications. The numbers of protein groups and precursors identified in each mixture (N1-N7) are shown, at the indicated on-column loading of total digest. Samples were analyzed using 5-minute microflow gradients and 7 msec MS/MS accumulation times. The bars indicate the proportion of identifications for each species (human – blue; yeast – orange; E. coli – green).

RESULTS

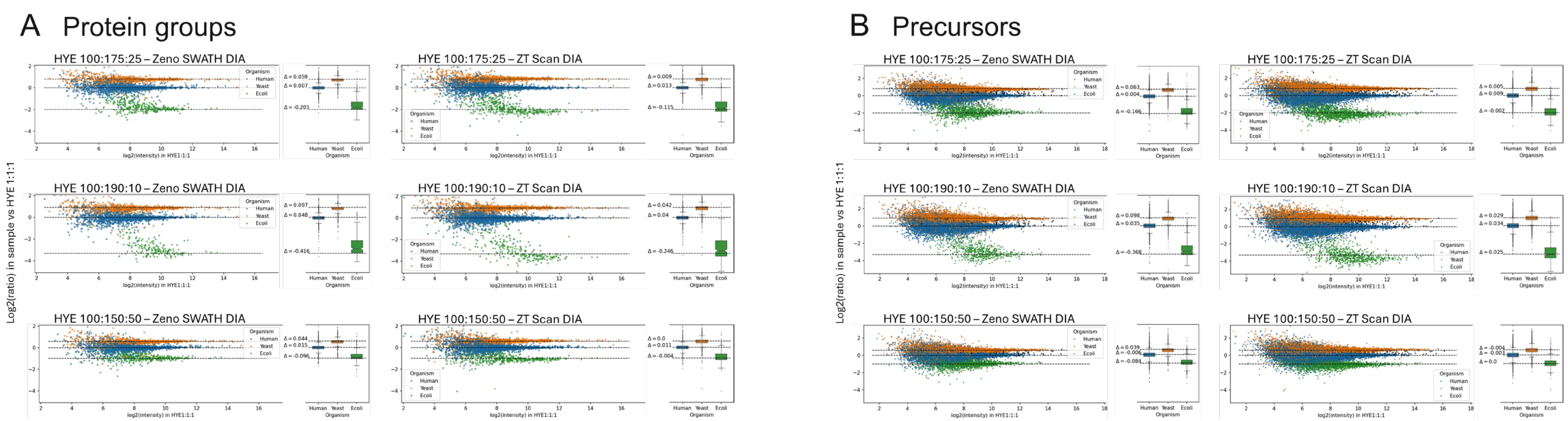


Figure 4. ZT Scan DIA improves LFQ accuracies compared to Zeno SWATH DIA. Comparisons between Zeno SWATH DIA and ZT Scan DIA for label-free quantitation of protein groups (A) and precursors (B) from varying mixtures of HYE (human/yeast/E. coli) species digests are shown. Injections of 50 ng were made using 5-minute microflow gradients, using either Zeno SWATH DIA or ZT Scan DIA with 7 msec MS/MS accumulation (i.e. dwell) times. LFQ plots show Log2 (mixture ratio) versus Log2 (intensity) for both protein groups and precursors, with color coding for each species indicated. The box-and-whisker plots indicate the quartiles and median values for each ratio, with the delta between the expected and observed ratios indicated.

RESULTS

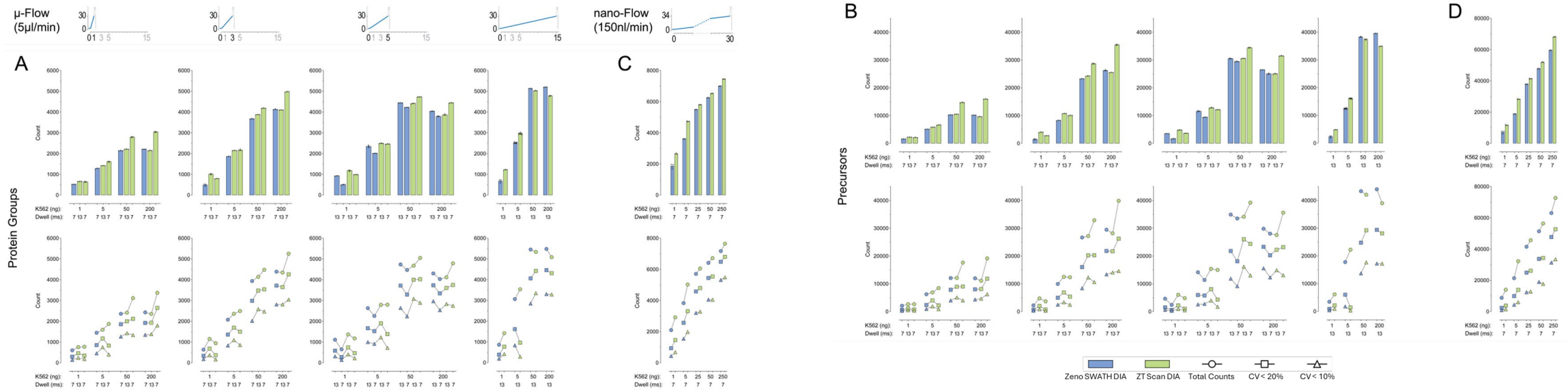


Figure 5. ZT Scan DIA improves the quantitative identifications of protein groups and precursors. Human K562 digest was analyzed using either Zeno SWATH DIA or ZT Scan DIA, with the on-column loading and chromatographic regime (microflow or nanoflow) indicated. The MS/MS accumulation time (i.e. dwell time, in milliseconds) for each experiment is shown. The resulting protein group and precursor identifications are shown in the bar graphs. The dot plots show the total identifications as well as the numbers identified with a coefficient of variation (CV) of either 20% or 10%. ZT Scan DIA is shown to improve the number of quantitative identifications, particularly for low on-column loadings or for fast gradient separations.

CONCLUSION

- ZT Scan DIA improves the numbers of protein groups and precursors identified and quantified in complex mixtures over discrete-window DIA methods, particularly when analyzing lower sample loadings and with faster LC gradient separations.
- ZT Scan DIA improves the accuracy of label-free quantitation of protein groups and precursors over discrete-window DIA methods, as demonstrated using LFQ experiments with human/yeast/E. coli mixtures.
- ZT Scan DIA methods are easy to set up and improve proteomics performance over a range of on-column loadings, sample complexity, and LC gradients, making them ideal for high-throughput biomarker research.

REFERENCES

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