Extending the limits of quantitative proteome profiling with data-independent acquisition

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Introduction

The data-independent acquisition (DIA) approach has recently been introduced as a novel mass spectrometric method that promises to combine the high content aspect of shotgun proteomics with the reproducibility and precision of selected reaction monitoring. Here we evaluate, whether SWATH-MS type DIA effectively translates into a better protein profiling as compared to the established shotgun proteomics.

Experimental Summary

HEK-293 and the 12 non-human spike in proteins were cells were prepared using Biognosys' Sample Preparation Kit according to manufacturers specifications. The samples were desalted using C18 macrospin columns from The Nest Group. The Biognosys' HRM Calibration Kit, was added to all of the samples according to manufacturer's instructions (required for the DIA analysis using Biognosys' Spectronaut).

Mass spectrometry

1 µg of the samples was analyzed on a self-made analytical column (75 µm x 30 cm) packed with 3 µm Magic C18AQ (Bruker) at 50°C, using an Easy-nLC II connected to a Q Exactive mass spectrometer (Thermo Scientific). The peptides were separated by a 2 h linear gradient of from 5 to 35 % ACN with 0.1 % formic acid at 300 nl/min. For DDA acquisition, the "fast" method from Kelstrup was used with the following alterations (Kelstrup et al.). The full scan was performed between 400-1,220 m/z. The HRM DIA method consisted of a survey scan at 35,000 resolution from 400 to 1,220 m/z (AGC target of $5*10^6$ or 120 ms injection time). Then, 19 DIA windows were acquired at 35,000 resolution (AGC target $3*10^6$ and auto for injection time).

Data Analysis

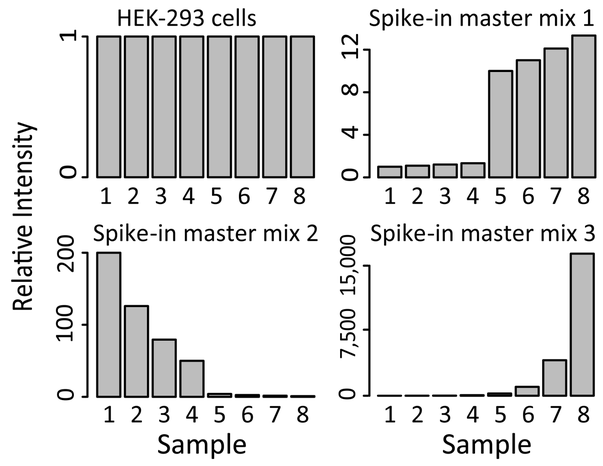
The DIA data were analyzed with Spectronaut, a mass spectrometer vendor independent software from Biognosys. The default settings were used for the Spectronaut search. The DDA spectra were analyzed with the MaxQuant analysis software using default settings with the following alterations (Kelstrup et al.). The minimal peptide length was set to 6 amino acids. The DDA files were searched against the human UniProt fasta database, the spike in proteins and the Biognosys iRT peptide sequences. The identifications were filtered to satisfy FDR of 1 % on peptide and protein level.

Statistical Comparisons

For both DDA and DIA, the log2-intensities of the peptides were summarized across all the samples and runs, and separately for each protein, in a linear mixed model implemented in MSstats (Choi et al.).

Figure 1: Design of the HRM and shotgun proteomics comparison experiment

The "Profiling Standard Sample Set" consisted of eight samples with a constant background (HEK-293 cells) and 12 non-human proteins spiked in three master mix groups. Master mix 1 and 2 were spiked in at small concentration changes on two concentration ranges. The master mix 3 was spiked in as a four fold dilution series. The lowest concentration was set to the value 1.



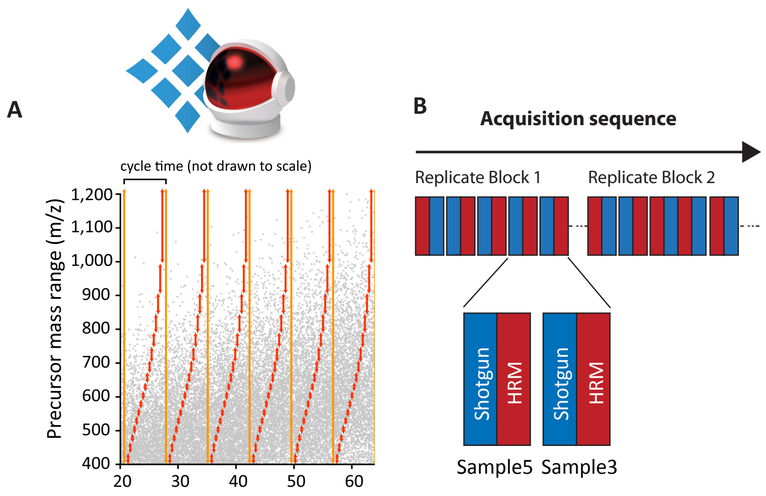
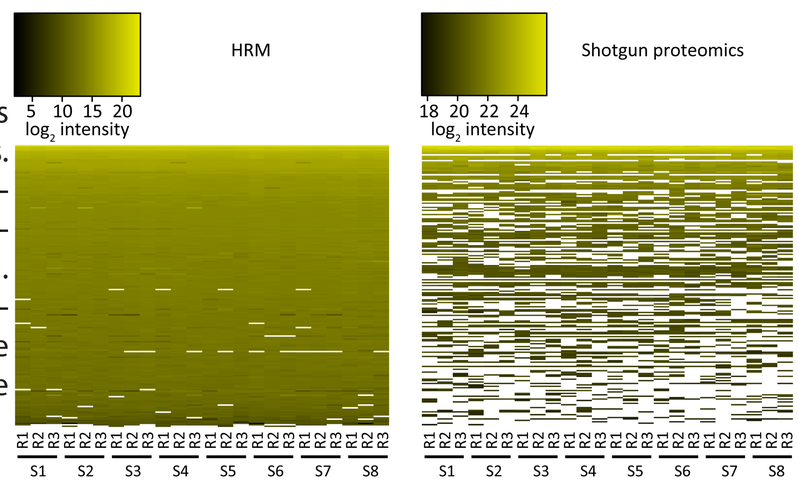


Figure 2: Novel DIA method and randomized acquisition

A The novel SWATH-MS type DIA method consisted of one survey scan and 19 DIA windows with adapted swath size to the precursor density implemented on a Thermo Scientific Q Exactive mass spectrometer. **B** Spectral acquisition of the "Profiling Standard Sample Set" was performed in shotgun proteomics and HRM mode in a block-randomized manner. This setup was chosen to avoid any bias.

Figure 3: Comparison of reproducibility of identification HRM vs Shotgun proteomics

200 peptides were randomly selected from all the peptides that were identified by both HRM and shotgun proteomics. The selected peptides were ordered in a heat map vertically by intensity and horizontally by sample. White areas indicate missing values of the peptide in the respective run. Over the whole data set, the reproducibility of HRM in peptide detection was above 98 % resulting in quasi complete data sets compared to 49 % of shotgun proteomics. Feature alignment was not used.



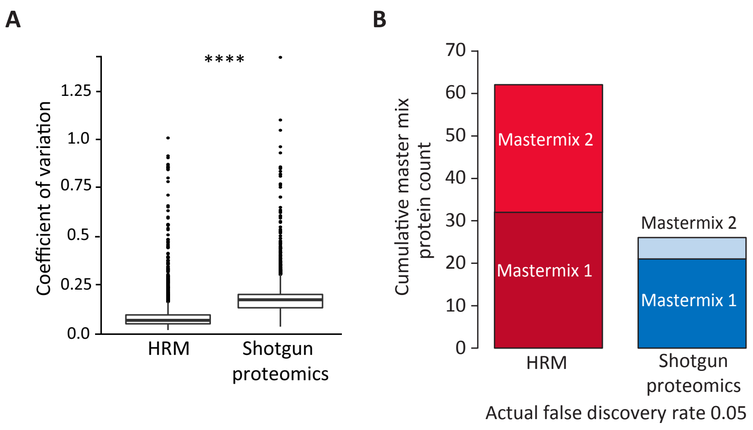
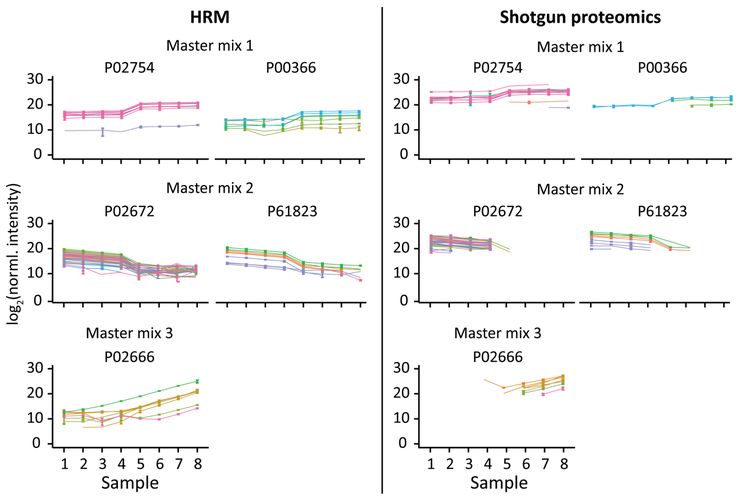


Figure 4: Quantitative Comparisons

A Coefficients of variation of 4,360 peptides from the background, which were identified in both shotgun proteomics and HRM, and quantified in all the 24 runs. The box plots show significant difference for HRM and shotgun proteomics (**** t-test, p-value < 0.0001). **B** The cumulative detection of spike in proteins of the 28 pairwise comparisons, after setting the cutoff of the actual FDR to 0.05 (the number of false positives divided by the number of candidates, from the ground truth).

Figure 5: Profiles of differentially abundant spike in proteins

Peptide profiles of five proteins of the three master mixes were visualized. The average and standard deviation of the normalized intensities per sample were plotted for the indicated proteins in HRM and shotgun proteomics. Only the significantly identified values are plotted. Note, that the HRM approach had better coverage of identification for the low concentration ranges.



Conclusions

- We implemented a novel DIA method on the widely used Orbitrap platform and used retention time normalized (iRT) spectral libraries for targeted data extraction using Spectronaut.
- Using a controlled sample set, we show that HRM outperformed shotgun proteomics both in the number of consistently identified peptides across multiple measurements, and quantification of differentially abundant proteins.
- Our findings imply that DIA should be the preferred method for quantitative protein profiling.

References:

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