DIA and Spectronaut: Comprehensive and precise proteome profiling

Oliver M. Bernhardt[1]; Roland M. Bruderer[1]; Tejas Gandhi[1]; Yue Xuan[2]; Lukas Reiter[1]

[1]Biognosys AG, Zurich, Switzerland
[2]Thermo Fisher Scientific, Bremen, Germany

Introduction

In recent years the proteome coverage using shotgun proteomics has steadily increased. Low complex proteomes, such as the *E. coli* or yeast proteomes, can be measured almost completely in a single LC-MS measurement. For relative quantitation, the label free approach has recently gained in popularity mainly due to its simplicity. However, this approach has been limited by the semi-stochastic nature of shotgun proteomics which leads to a large number of missing values, especially if many conditions are measured. Even though MS1 alignment attenuates the missing value problem, it is difficult to control the reliability of identification with this approach. Further, low intensity signals in MS1 often show interferences which lowers the precision of relative quantitation. Data independent acquisition (DIA) has promised to solve the missing value problem. By using wide precursor windows, DIA consistently measures all precursors that are above the limit of detection. For DIA data analysis we have developed the Spectronaut software. Using Spectronaut, we get more identifications in a single LC-MS measurement as compared to shotgun proteomics. Further, this approach resulted in quasi gap-free quantitation matrices without alignment and higher precision of quantitation as compared to shotgun proteomics.

Methods

Protein extracts from human HEK-293 or HeLa cell line cultures were tryptically digested, spiked with the Biognosys HRM Kit and measured on a Q Eactive or Q Exactive HF (Thermo Fisher Scientific). All samples were measured using a 2h gradient. Targeted analysis of DIA data was performed using Spectronaut (Biognosys). Spectral libraries were generated with MaxQuant and Spectronaut. Among the information in the spectral library are the precursor and fragment ion m/z, the indexed retention time of the peptide (iRT) (Escher et al. 2012) and relative fragment ion intensities. False discovery rates are estimated using the mProphet method (Reiter et al. 2011). To increase the precision of quantitation the interference detection algorithm as implemented in Spectronaut was used. Fragment ion signals not following the consensus elution profile are consistently removed from quantitation across all conditions.

Summary

Spectronaut is a fast and easy to use software for the targeted analysis of DIA data and quantitative proteome profiling. The hallmarks of the resulting data are high proteome coverage, very precise proteome profiling and nearly gap free quantitation matrices without the use of alignment algorithms.

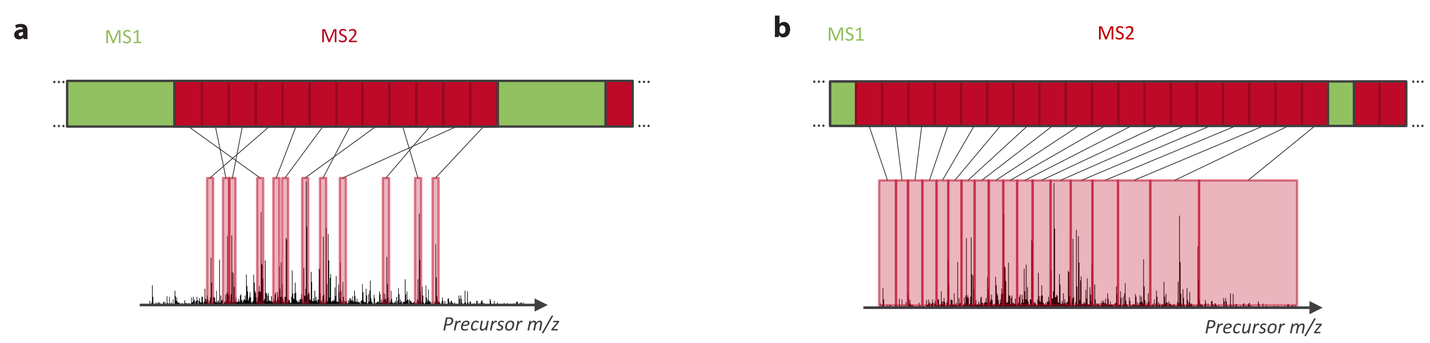

Figure 1. Schematic illustration of a typical DDA/shotgun (**a**) and DIA (**b**) method as implemented for instance on a Q Exactive mass spectrometer. (**a**) In a DDA method the mass spectrometer typically cycles between a high resolution MS1 full scan (e.g. 70k resolution) and a number of data dependent MS2 scans (e.g. 17k resolution) depending on the precursor ions detected in the MS1 scan. (**b**) In a DIA method the mass spectromter typically cycles between a high resolution MS1 full scan (e.g. 70k resolution) and a number of consecutive MS2 scans covering the full precursor range.

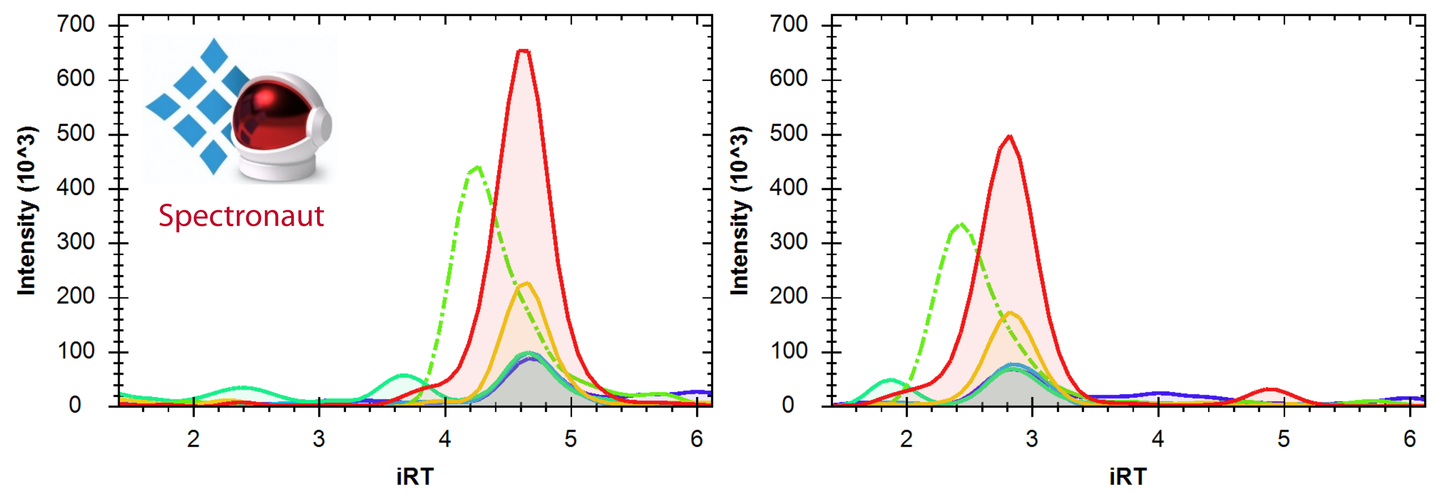

Figure 2. Extracted ion chromatograms (XIC) on MS2 level (fragment ions) for the peptide LLLDQEQK are shown in two LC-DIA runs. In this example the fragment ion y7+ (green dotted line) shows an interference. Spectronaut automatically detects the interference because the XIC of y7+ is not following the consensus elution profile. With the automatic interference correction turned on, Spectronaut excludes the XIC of y7+ consistently from quantitation across all the runs.

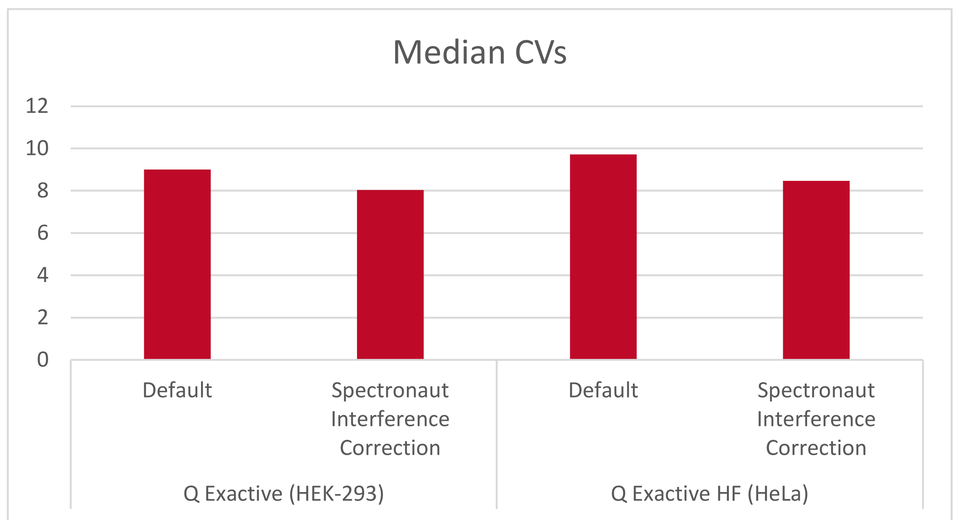

Figure 3. Median CVs across triplicates with and without Spectronaut interference correction for a HEK-293 sample acquired on a Q Exactive (29'102 peptide precursors identified) and a HeLa sample acquired on a Q Exactive HF (74'807 peptide precursors identified). Median CVs are lower if the automatic interference correction is enabled in Spectronaut.

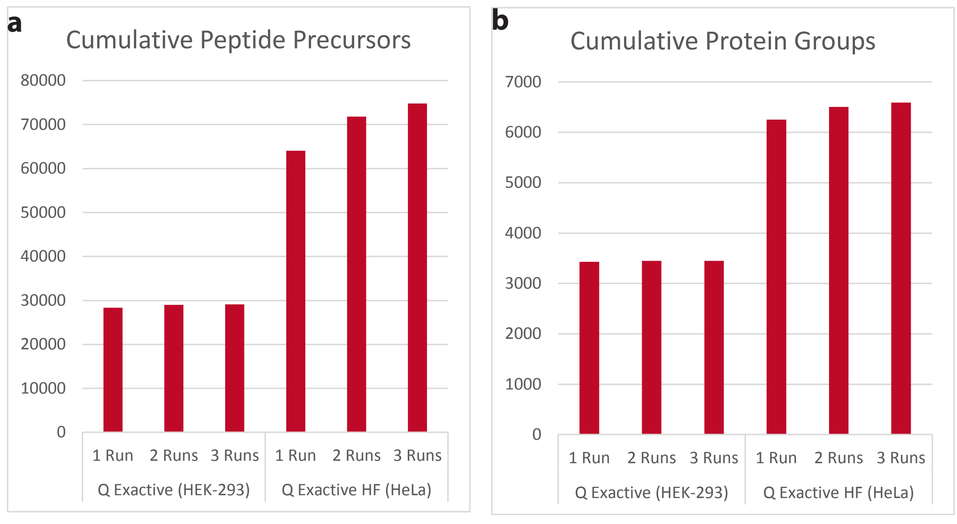

Figure 4. Number of cumulative peptide precursor (**a**) and protein group (**b**) identifications for triplicate 2h runs of a HEK-293 and HeLa sample acquired on a Q Exactive and Q Exactive HF respectively. Both instruments feature very high reproducibility with DIA. The Q Exactive HF identifies more than 6'000 protein groups, almost double the number of the Q Exactive.