BIOGNOSYS
NEXT GENERATION PROTEOMICS

APPLICATION NOTE

Comparison of Static and Dynamic Segments in Data-Independent-Acquisition (DIA) Experiments

SUMMARY

Data independent acquisition methods are based on the parallel acquisition of broad ranges of peptide precursors. The distribution of the precursor masses, however, is not equally distributed over the mass range. An obvious solution is to use broad windows where the precursor density is low and narrow windows where the density is high, instead of using static windows across the whole mass range.

What is the benefit of using an optimized dynamic window method compared to using one simple standard method with static windows for all samples? In this application note we try to answer this question and give recommendations for the selection of optimal mass ranges on Orbitrap instruments.

Main findings:

- Dynamic and static DIA window methods perform similarly in terms of the number of quantified proteins and quantitative precision
- This finding is independent of DIA analysis strategy (spectral library based approach or directDIA™)
- For static segments, a mass range from 350 to 1,200 m/z is recommended for a Q Exactive HF mass spectrometer

INTRODUCTION, METHODS & RESULTS

INTRODUCTION

Data-independent acquisition (DIA) methods are becoming increasingly popular for comprehensive, precise and reproducible proteome quantification. The most commonly used DIA method was introduced by the Aebersold group with a set of 24 static DIA segments at a width of 25 Thomson (Th)[1].

As the precursor distribution across the mass range is not homogenous (***Fig. 1A***), it has been proposed to adjust the width of the DIA segments according to the density distribution of the precursor m/z[2]. The rationale behind this approach is to reduce the complexity of the DIA spectra in highly populated mass ranges and to avoid 'empty' DIA spectra in loosely populated mass ranges. An evenly distributed number of precursors per DIA window should thus lead to the highest number of quantified precursors and subsequently of proteins.

In this application note we compare a dynamic DIA window method, which was optimized on the m/z distribution of a Hela digest, to four different static DIA window methods on an Orbitrap instrument.

Figure 1: . DIA Window Design

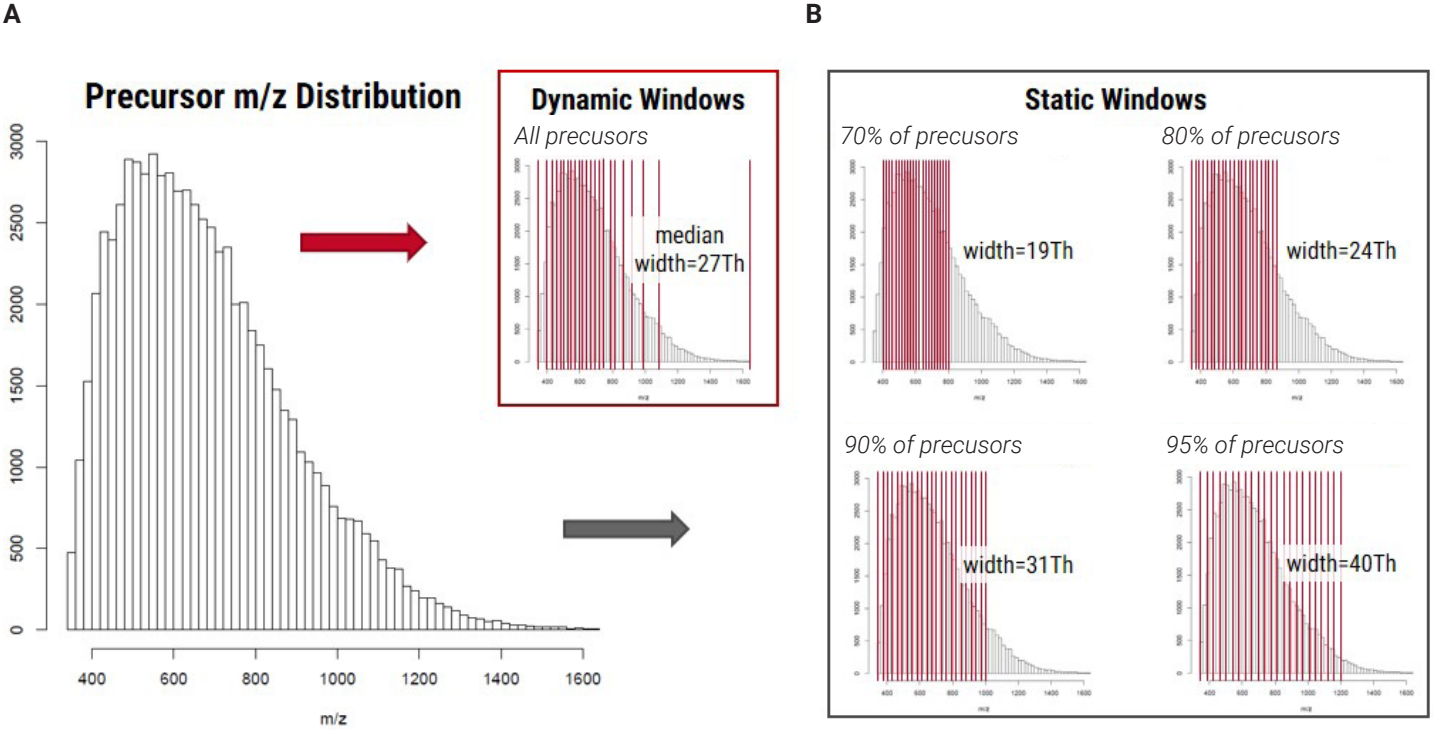

Figure 1. A. Precursor m/z distribution of a Hela digest. B. DIA window design for dynamic and static DIA segments covering different mass ranges.

METHODS

Our aim was to evaluate whether it is beneficial to adjust the DIA window width to the precursor distribution or whether the easy to implement static DIA window methods perform comparably. For the static DIA window methods, the mass ranges were reduced compared to the dynamic DIA window method to use smaller static DIA segments.

The four methods covered 70%, 80%, 90% or 95% of the precursors used for the variable method. The data was analyzed with a classic spectral library based approach, as well as with our newly introduced directDIA™ approach.

A Hela sample was prepared according to Biognosys' Sample Preparation Kit Pro and spiked with iRT peptides (Biognosys) according to the instructions. Two µg of the peptide mixture was separated by a non-linear 2h gradient on an Easy nLC1200 (Thermo Fisher Scientific) coupled online to a Q Exactive HF mass spectrometer (Thermo Fisher Scientific).

We set the number of DIA segments to 22 for all methods to achieve on average 5-7 data points per peak (FWHM*1.7). A dynamic DIA window method was calculated based on the precursor m/z distribution of the same sample with an overlap of 1 Th.

The mass ranges for the static DIA window methods were determined based on the same m/z distribution to include 70% (400-800 Th), 80% (350-850 Th), 90% (350-1,000 Th) or 95% (350-1,200 Th) of the precursors (***Fig. 1B***).

For the static window DIA methods, the mass ranges were separated into 22 equidistant DIA segments with an overlap of 1 Th. DIA scans were acquired with a resolution of 30k. All samples were analyzed in technical triplicates. Data were analyzed in Spectronaut Pulsar with a Hela spectral library (provided in Spectronaut Pulsar) and by directDIA™. All results were filtered by a FDR of 1% on precursor and protein group level.

RESULTS & DISCUSSIONS

RESULTS

The comparison of four static window methods and a dynamic window method optimized for a specific sample showed, as expected, that the use of the latter method resulted in the highest overall number of quantified precursors (58,455) (***Fig. 2A***). The static window methods led to lower number of quantified precursors especially when using limited mass ranges.

For the majority of proteomic experiments the number of quantified proteins is more important than the number of precursors. Therefore, we examined closer how the different methods performed on the protein group level. The dynamic range method led to the most proteins being identified (5,144), but the static window methods performed equally well, all within 99% of the protein numbers achieved with the dynamic range method ***(Fig. 2B***).

We also investigated how different methods influenced the number of identified peptides per proteins (***Fig. 2C***). Around 50% of the proteins were very confidently quantified with more than seven peptides per protein. As expected, this percentage was slightly higher with the methods that covered a larger mass range. All methods resulted in ~10% single-peptide-identifications.

These findings indicate that by extending the mass range more peptides per proteins are quantified, rather than more proteins with a very low number of peptides. The precision of quantification was not influenced by different methods and was comparably low on precursor (CV <8%) and protein level (CV <5%) for technical run replicates.

Figure 2. Spectral-Library-Based Analysis

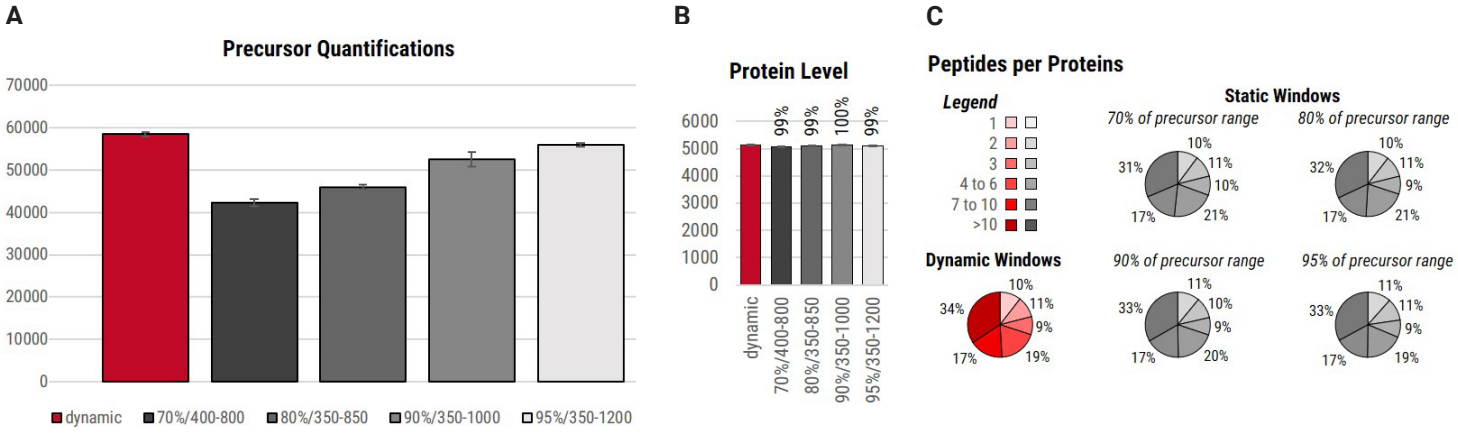

Figure 2. A. Overview of quantified precursors for each DIA method for the whole mass range as well as for the mass ranges the different methods were limited to. B. Overview of quantified protein groups for each DIA method for the whole mass range. C. Overview of the number of quantified peptides per protein (unique peptide sequences).

Figure 3. directDIA™ Analysis

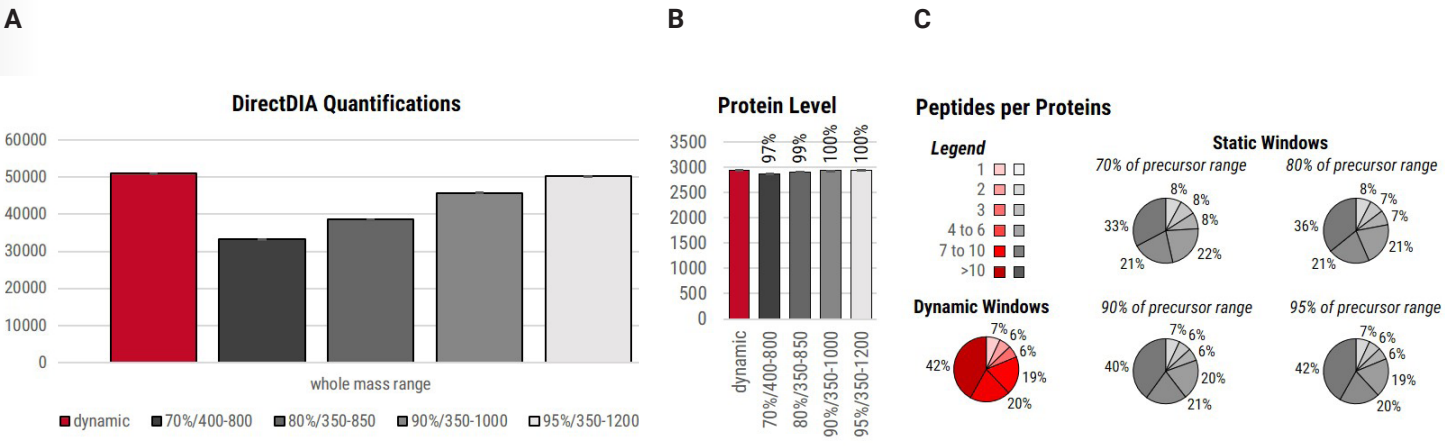

Figure 3. A. Overview of quantified precursors for each DIA method for the whole mass range as well as for the mass ranges the different methods were limited to. B. Overview of quantified protein groups for each DIA method for the whole mass range. C. Overview of the number of quantified peptides per protein (unique peptide sequences).

Additionally, we also analyzed the data with our newly introduced directDIA™ approach which enables direct database search of DIA without the need to generate a spectral library. Here as well, the dynamic window method resulted in the largest number of quantified precursors (50,996) (***Fig. 3A***). Static window method covering 95% of the precursors performed equally well (50,721 precursors; 99%), whereas again the other static window methods resulted in lower numbers of quantified precursors.

Similarly to the spectral library based analysis, the number of quantified proteins obtained with the directDIA™ approach was comparable between the dynamic window and static window methods (***Fig. 3B***).

In regards to the number of identified peptides per proteins, the directDIA™ approach led to only ~8% single-peptide-identifications. Furthermore, using the directDIA™ around 60% of the proteins were identified with more than 7 peptides per protein (***Fig. 3C***). Similarly to the spectral library based approach, this percentage was higher with methods that covered a larger mass range.

The quantitative precision of the directDIA™ was also comparable between different methods (precursor level CV <7%; protein level: CV <4%).

CONCLUSIONS & REFERENCES

CONCLUSIONS

- Dynamic and static DIA window methods perform similarly in terms of the number of quantified proteins and quantitative precision under the conditions tested
- This finding is independent of DIA analysis strategy (spectral library based approach or directDIA™)
- The limited mass range for the static window methods led to fewer identified peptides per protein, but not to more sinlge-peptide-identifications
- For static segments, a mass range from 350 to 1,200 m/z is recommended for a Q Exactive HF mass spectrometer

REFERENCES

1. Gillet, L. C., Navarro, P., Tate, S., Rost, H., Selevsek, N., Reiter, L., … Aebersold, R. (2012). Targeted Data Extraction of the MS/MS Spectra Generated by Data-independent Acquisition: A New Concept for Consistent and Accurate Proteome Analysis. Molecular & Cellular Proteomics, 11(6), O111.016717-O111.016717. http://doi.org/10.1074/mcp.O111.016717

2. Bruderer, R. et al. Optimization of Experimental Parameters in Data-Independent Mass Spectrometry Significantly Increases Depth and Reproducibility of Results. Molecular & Cellular Proteomics 41, (2017). http://www.mcponline.org/content/early/2017/10/25/mcp.RA117.000314.abstract

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