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NEXT GENERATION PROTEOMICS

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DirectDIA, a Versatile Tool to Answer Many Proteomics Questions

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Introduction

In recent years, three main flavors of data-independent acquisition (DIA) analysis have emerged as the dominant strategies. The classical approach relies on creating a deep sample specific library using data-dependent acquisition (DDA). However, this significantly increases the overhead for doing DIA. So, two “library-free” approaches have become popular. The first approach relies on creating an in-silico library from a full proteome digest by prediction using a deep neural network. The second involves a spectrum-centric analysis to create a library directly from the DIA runs as featured in directDIA or DIA-Umpire (Tsou et al. 2015). Here we want to discuss the benefits and weaknesses of these approaches and highlight directDIA as a versatile solution for a variety of challenging experimental circumstances.

Methods

For this analysis we look at several common proteomics experiment types and discuss the strength and weaknesses of each workflow, respectively. We benchmarked Spectronaut using directDIA against the classical DIA analysis approach for depth of proteome coverage and quantitative accuracy. We further highlight the versatility of directDIA by showcasing its ability to analyze experiments which remain highly challenging for other library free approaches like peptide centric searches using in-silico libraries. Those include an unspecific search of immunopeptidomics samples and Phospho-PTM localization experiments with several variable modifications.

Preliminary Data

First, we wanted to look at the quantitative capabilities of directDIA using controlled quantitative benchmark experiments (CQE). For that we analyzed several sample sets of varying gradient length and MS platforms. Notably, directDIA is most competitive in larger experiments with biological diversity and would therefore have a disadvantage in short LFQ-Bench type experiments. Regardless of that, not only do we achieve very comparable numbers of identifications compared to deeply fractionated spectral libraries but can surpass them in quantitative precision as well as data completeness in controlled quantitative experiments.

In order to further investigate the strengths and weaknesses of the different analysis workflows we took to more challenging experimental setups. As a stand in for a PTM centric analysis we looked at a quantitative phospho proteomics benchmark published by Bekker-Jensen et al. We show that directDIA is producing better quantitative results for phospho site localization

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compared to DDA libraries. Further, it is currently the only library free DIA approach that can deal with this search space.

Similarly challenging are experiments that require a non-specific search space. We analyzed a set of human immunopeptidomics samples using both a classical library search as well as directDIA. The resulting data from both workflows showed high levels of agreement on their omega anchor profile and a comparable peptide length distribution.

We show that the spectrum-centric analysis workflow (directDIA) implemented in Spectronaut supports all these scenarios and that it also delivers good reproducibility, data completeness, and quantification as well as an overall comparative depth of proteome coverage.

Novelty aspect

Characterization of peptide- and spectrum-centric DIA analysis approaches on different experiment types and discussion about inherent strengths and weaknesses.