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Proteoform Mapping Calls for a Combined Effort From Bottom-Up and Top-Down Proteomics

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ABSTRACT

Proteomics has demonstrated that each protein consists of various proteoforms that provide an additional layer of biological data next to gene-, transcript-, or protein expression levels. As a result, proteome analyses are increasingly being complemented with proteoform maps. Identification and quantification of the type, site, and dynamics of a proteoform modification contribute to a better understanding of human biology. Testing the hypothesis that proteoform-resolved data can provide novel tools in precision medicine requires robust and high-throughput proteoform measurements. The prime candidate for this purpose is top-down proteomics (TDP), commonly performed with mass spectrometry. In this Special Issue: Top-Down Proteomics, Fornelli et al. report a comparative analysis of various SDS-removal methods that are needed for proteoform mapping in TDP experiments. Their work provides technical guidance on an important aspect of the TDP sample preparation process.

In this issue of PROTEOMICS, Fornelli et al. highlight the sample preparation part for top-down proteomics (TDP) and report on a comparative analysis of proteoform clean-up methods [1]. Proteoform analysis became intertwined with TDP from the moment the term "proteoform" was introduced [2]. The TDP consortium encouraged the field to optimize and innovate TDP strategies aiming for comprehensive proteoform maps and make workflows available for a broader proteomics community, including sample preparation, proteoform separation, MS instrumentation, and computational platforms and software [3, 4]. The study from Fornelli et al. contributes to improvement of TDP sample preparation by addressing the detergent clean-up. Starting from complex cell lysates from *Escherichia coli* as well as Jurkat cells, the authors systematically compare various proteoform clean-up methods followed by TDP proteoform analysis using state-of-the-art MS instrumentation [5]. Proteoforms in the cell lysates are upfront separated and collected in intact form using passive elution from polyacrylamide gels (PEPPI platform). SDS-removal is crucial for identification of proteoforms, and methanol-chloroform-water (MCW) precipitation is commonly used for this purpose. The authors benchmark four commercial SDS cleanup alternatives to MCW precipitation with regard to efficiency and reproducibility of SDS removal and intact proteome coverage. The number of identified proteoforms is thoroughly evaluated for potential bias in proteoform retention based on physicochemical properties and the types of PTMs present. Moreover, ease of use and the costs of the commercial kits are overviewed, resulting in practical guidance regarding each method's trade-offs.

In the pursuit of precision medicine, protein phenotypes are foreseen that, combined with pattern recognition tools, exhibit great potential in guaranteeing safe and accurate test results for patient management. To that end, proteomics has greatly contributed to a systems-level understanding of human biology by identifying molecular pathways that underlie disease pathophysiology. Proteomics has also elucidated that each protein is a collection of various proteoforms that are more closely linked to biological function than gene-, transcript- or protein expression levels [2, 6]. It is therefore assumed that proteoforms provide an additional structural layer to protein quantities with potential for further patient stratification [7]. Whether or not proteoform-resolved data holds promise for clinical purposes needs to be

determined from evaluations of patient cohorts that require robust and high-throughput measurements. In a TDP experiment a protein is characterized (generally by mass spectrometry) in its intact form to provide a detailed view of its post-translational modifications (PTMs), genetic variations, and alternative splicing variants. However, from TDP results, it also become clear that additional tools and strategies are needed to comprehensively characterize proteoforms within a certain proteome. This is exemplified by membrane- and high molecular weight proteins (antibodies!), as well as for the quantification of the type, site, and dynamics of a proteoform modification. These examples require complementary middle-up or bottom-up strategies, such as for glycoproteoforms that are decorated with complex PTMs [8, 9]. It is interesting to notice that modified peptide identifications from bottom-up proteomics experiments are increasingly referred to as peptidoform identifications, similar to proteoform terminology [10]. With regard to the MS-based quantification performance, the intact protein level is more straightforward than peptide-based quantification. Next to detailed structure characterization, TDP mass spectra provide signal intensities for each charge state of a proteoform that can be normalized and used for relative quantitative purposes. However, the relative quantities of peptidoforms also provide an attractive alternative for proteoform quantification. Concerted efforts from both top-down and bottom-up proteomics experts are foreseen, which will allow testing the hypothesized clinical utility of proteoform-resolved data [8, 11, 12]. In the aforementioned proteoform clean-up study, the authors discuss that MS-based TDP proteoform analysis is currently limited to proteins smaller than 30 kDa due to MS-signal intensity spread. A second challenge concerns the sequence coverage that turns lower for larger proteoforms as a result of less backbone fragmentation, thus affecting the confidence of the identifications. Collectively, these limitations and considerations call for top-down and bottom-up proteomics to move forward together to accelerate progress in comprehensive and quantitative proteoform analysis.

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