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Applications and Developments in Targeted Proteomics: From SRM to DIA/SWATH

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Editorial for special issue 'Applications and developments in targeted proteomics: from SRM to DIA/SWATH'

The adaptation of targeted data acquisition from small molecule analysis to bottom-up proteomics in the form of selected reaction monitoring (SRM) approximately a decade ago was initially motivated by the need for robust and sensitive quantification of proteins in both basic life science research and translational medicine. This need was precipitated by limitations with respect to quantification, repeatability and reproducibility that were associated with data dependent acquisition (DDA) caused by the now well described issues surrounding stochasticity of precursor selection¹. Over the years, the capabilities of targeted data acquisition by SRM², and its more recently implemented cousin parallel reaction monitoring^{3,4} (PRM), have developed remarkably in both instrumentation and software, enabling robust and sensitive quantification of discrete sets of proteins across large sets of samples. Further, the quality of the quantitative data available from targeted approaches and the robustness of the methods have enabled highly successful applications in a variety of arenas and this has led to broad community acceptance of the utility of targeted proteomics⁵.

While targeted data acquisition by SRM or PRM has been successful, the methods are also limited in utility for some applications. Most obviously, data is acquired for a set of proteins, defined prior to acquisition and once acquired, there is no possibility to re-mine the data to obtain information for other targets. In principle, data independent acquisition⁶ (DIA) alleviated the requirement to define a set of proteins prior to acquisition and could expand to quantify larger sets of proteins. The introduction of targeted data analysis to DIA, implemented as SWATH-MS⁷, drew on analysis principles similar to those developed for data from targeted acquisition. This marked a turning point in DIA that facilitated the generation of data that maintained the favorable quantitative characteristics of targeted data acquisition, while significantly expanding the coverage of proteins compared to SRM or PRM. This advance has led to significant method developments in this space, in parallel to successful applications in many research areas.

The term DIA has broadly referred to a range of deterministic acquisition methods⁸ accompanied by data analysis strategies⁹ which fall into either the peptide-centric analysis category^{10,11}, utilizing targeted data analysis similar to SWATH-MS, or the spectrum-centric data analysis category, that are conceptually more similar to classical database search strategies. To capture the range of approaches, we have chosen to use the inclusive term DIA/SWATH in the title of this special issue to indicate that the focus here is on DIA strategies which draw on the concepts and analysis strategies which are characteristic of targeted proteomics (as in SWATH-MS). Furthermore, applications in DIA/SWATH may use terms borrowed from targeted data acquisition such as "targeted assay" or "library" to describe a set of analyte parameters used for data extraction from DIA data. Additional discussion could examine and perhaps refine these terms in the context of DIA, for example, similar to a recent community effort which proposed categorization of assays of various stringency levels for different types of basic science or clinical applications in targeted MS measurements¹². We hope this special issue will stimulate such a conversation in this area regarding terminology.

The goal of this special issue was to sample the breadth of applications currently being pursued using these techniques as well as to represent ongoing technical developments. In the first section of the volume, 7 papers describe *applications* in the scope of the special issue. In the second section, 6 papers *review* and *compare* different techniques and discuss differences between them. And in the third and final section, 5 papers suggest various *developments* in acquisition and data analysis.

With these applications and developments in mind we might ask what will the future hold for targeted proteomics and related DIA-based methods? With the rapid increase in the scanning speed

of high resolution mass spectrometers the lines between approaches such as PRM, DDA, and DIA/SWATH will begin to blur and a unified acquisition method could emerge in which high resolution MS2 spectra are acquired deterministically with narrow precursor isolation windows and comprehensive sampling of the precursor space. With such comprehensive and high quality data it seems likely that the focus of discussion will be whether the data should be analyzed by peptide-centric approaches or by spectrum-centric approaches. With the availability of unprecedented data sets generated by these methods, the focus of quantitative proteomics might also partially shift away from technical aspects towards the most innovative applications of the powerful technology to generate new biological and clinical knowledge.

What then is the future for targeted proteomics using low resolution mass spectrometers (e.g SRM)? The sensitivity, relatively low cost and robustness of triple quadrupoles will likely keep this method in favor for routine applications where discrete sets of proteins need to be measured in large numbers of samples. In particular, translating targeted assays into analytical tier 1 assays for potential future applications in clinical research remains an attractive prospect¹². In broader terms, targeted proteomics using SRM/PRM for basic research purposes is also likely to persist as the level of sensitivity and selectivity achievable may be still favorable compared to DIA-based methods.

We would like to thank all of the authors who contributed articles to this special issue, the reviewers who provided critical feedback, and the editors of PROTEOMICS for making this special issue possible. Based on the enthusiastic response to the call for submissions to this special issue, one point is clear: The interest in continuing to develop and apply methods in this area is increasing and there are surely exciting times ahead. We speculate that the bright line that has separated targeted proteomics from discovery proteomics will begin to fade leaving only a broad quantitative proteomics method for life science research which is robust, reproducible and comprehensive¹³. We hope that the collection of papers in this special issue will help to underscore the power and potential of this arena of quantitative proteomics.

Guest editors: Ruedi Aebersold, Ariel Bensimon, Ben C. Collins, Christina Ludwig, Eduard Sabido

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