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Targeted protein identification, quantification and reporting for high-resolution nanoflow targeted peptide monitoring☆,☆☆

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ABSTRACT

Mass spectrometry-based targeted proteomic assays are experiencing a surge in awareness due to the diverse possibilities arising from the re-application of traditional LC-SRM technology. The FDA-approved quantitative LC-SRM-pipeline in drug discovery motivates the use to quantitatively validate putative proteomic biomarkers. However, complexity of biological specimens bears a huge challenge to identify, in parallel, specific peptides and proteins of interest from large biomarker candidate lists. Methods have been devised to increase scan speeds, improve detection specificity and verify quantitative SRM-features. In contrast, high-resolution mass spectrometers could be used to improve reliability and precision of targeted proteomics assays. Here, we present a new method for identifying, quantifying and reporting peptides in high-resolution targeted proteomics experiments performed on an orbitrap hybrid instrument using stable isotope-labeled internal reference peptides. This high precision targeted peptide monitoring (TPM) method has unique advantages over existing techniques, including the need to only detect the most abundant product ion of a given target for confident peptide identification using a scoring function that evaluates assay performance based on 1) m/z-mass accuracy, 2) retention time accuracy of observed species relative to prediction, and 3) retention time accuracy relative to internal reference peptides. Further, we show management of multiplexed precision TPM-assays using sentinel peptide standards. This article is part of a Special Issue entitled: Proteomics from protein structures to clinical applications (CNPN 2012).

Abbreviations: TPM, targeted peptide monitoring; MRM, multiple reaction monitoring; SRM, selected reaction monitoring; RT, retention time; IT, ion trap; FT, Fourier transform; LOD, limit of detection; SLG, Serine–Leucine–Glycine; amu, atomic mass units; ppm, parts-per-million; HRIS, High-Resolution Identification Score.

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☆☆ Brief communication: We describe the development and application of a high-resolution, multiplexed quantitative nLC-TPM pipeline, and validate the specificity of such assays for detecting peptides of selected microRNA protein targets in a proteolytic digest of mouse cardiomyocyte soluble protein extracts.

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1. Introduction

Targeted proteomic technologies are amongst the most promising emerging methods for the discovery and validation of drug targets and for monitoring patient health on a system level. While successfully applied to a variety of biological problems in basic proteome research, targeted proteomics based on low-resolution mass spectrometer has fundamental drawbacks [1,2]. While LC-SRM analysis has been used for many decades to quantify drugs and metabolites, transferring this platform to the proteomics domain has faced obstacles due to the greater complexity of the proteome [3]. Limitations in speed and sensitivity hinder true assay multiplexing, and lack in specificity detecting analytes in complex sample matrices, renders low resolution SRM-feature identification and assay fidelity questionable [4]. Nevertheless, the introduction of new high-resolution instruments can markedly improve the accuracy of qualitative and quantitative analysis of small molecules in biological matrices [5,6]. Interestingly, the small molecule research community had been much faster in exploiting this potential in high-resolution SRM-assays and already has undertaken evaluation studies to test the promised benefits [7–9]. This groundwork has shown that a resolving power lower than 15,000 full width half maximum (FWHM) does not ensure sufficient specificity in targeted metabolomics assays. Conversely, the resolving power and mass stability of high performance instruments are recognized as highly beneficial in driving SRM small molecule assays with the highest possible precision, reproducibility, dynamic range, and detection limits. Yet analogous studies have been rare in the proteomic arena and have only just recently been attempted with the appearance of popular hybrid orbitrap instruments [10]. In addition to this, a few previously published works on targeted proteomics using workhorse ion traps reported acceptable quantitative accuracy [11,12] and linear dynamic range comparable to data obtained with triple stage quadrupole or Q-trap instruments for metabolites [13,14]. The benefit of trap-type instruments is that 1) multiple SRM-transitions (so called TPM-transitions) are obtained at no additional performance cost and 2) produce highly reproducible and laboratory-independent fragmentation patterns, which then can be used flexibly for more refined identification purposes further downstream of the analytical pipeline [15,16].

The main consideration of low resolution SRM-based proteomics was that in complex sample matrices upwards of 8 or more SRM-transitions need to be simultaneously monitored in order to confidently identify a particular peptide at any given retention time based on a relative intensity pattern match that is not influenced by the sample background (i.e. interferences) [16]. However, as highest assay sensitivity is achieved through the monitoring of only a single highly specific and abundant SRM-transition, assay performance and precision would seemingly benefit greatly from high-resolution instrumentation. In traditional small molecule SRM-assays, quantification is typically done using one specific and sensitive SRM-transition, called "quantifier", which itself is extensively validated for specificity, accuracy and precision while few further transitions may be used for validation as "qualifiers" or "identifiers" [17].

Moving clinical diagnostics towards a personalized precision medicine [18,19], the proteolytic background of individual patient specimens might differ largely. Hence, it would be desirable, depending on sample background, to be able to flexibly increase assay specificity and verify tentative identifications after completion of data collection, without reanalyzing or re-acquiring a sample. Thus, our opinion is, collecting all informative product ion information will be crucial for downstream sample investigation and precise personalized diagnosis in a system medicine setting [20]. Hyphenated ion trap-orbitrap instruments offer the unique advantage of a highly reproducible and controlled fragmentation process combined with high mass resolution, accuracy and precision to potentially achieve the most sensitive and highly specific LC-TPM-assays.

Here we show that such targeted high-resolution multiplexed TPM-assays are sensitive, quantitative and can be at least moderately multiplexed on an LTQ-Orbitrap-Velos mass spectrometer. Furthermore, we show that this approach is feasible for achieving confident protein identifications using only a single product ion feature, when accurate retention time information is known and heavy stable isotope reference peptides are used to find the correct target retention time window. We also show that further validation using all recorded product ions assists peptide validation of target ion species especially when unexpected contextual retention time shifts occur, depending on different sample matrices. To evaluate candidate matches, we have created a unified scoring function, which measures product ion m/z and retention time-accuracy and precision. We apply the new method to monitor spiked light and heavy peptides within the cytoplasmic proteolytic background of mouse cells that are predicted targets of microRNAs.

2. Methods

For clarity reasons, in this manuscript we use the IUPAC "SRM"-terminology for targeted proteomic assays performed on triple quadrupoles, i.e. when the second ion filter can be driven to statically select one single transition, but use the term "TPM", when all product ions are collected in an ion trap or an orbitrap and precursor ion m/z values were added in the MS method pre-acquisition and all product ions were detected after undergoing CID in the ion-trap and target precursor–product transitions then were software selected post acquisition.

2.1. Materials

Synthetic peptides in unpurified form were obtained from JPT Peptide Technologies, Berlin, Germany in unlabeled and stable isotope (C-terminal (13C15N) K and R amino acids) labeled form. Heavy peptides containing C-terminal (13C15N)-K and -R have a mass of +8 and +10 Da to their unlabeled peptide form. Each peptide was synthesized with the high-throughput SPOT-peptide synthesis method and contained a common tri-peptide (X-SLG) sequence tag at the C-terminus. All LC-MS solvents were HPLC grade (Avantor Performance Materials, JTBaker products, Center Valley, PA). Sequencing-grade trypsin was purchased from Roche, Mississauga, Canada. All other chemicals were obtained from SigmaAldrich, Mississauga, Canada. Luna-C18 (3 μm)

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