

SpikeTides™ - Proteotypic Peptides for Large-Scale MS-Based Proteomics

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Targeted proteomics, as an efficient and sensitive technology for protein identification and quantification, is dependent on the availability of custom peptides for assay development and absolute quantification. Current workflows rely on the use of peptides that are synthesized by labor-intensive resin-based solid-phase peptide synthesis approaches. In addition, absolute protein quantification requires heavily labeled peptides that must be quantified. The result is a high price per peptide, which often limits focus to a few selected targets and thus prohibits large-scale projects.

Introduction

SpikeTides™ are an innovative way to overcome these limitations. They are small-scale, inexpensive, heavily labeled or non-labeled, and/or absolutely quantified peptides for single reaction monitoring (SRM) and multiple reaction monitoring (MRM).

Targeted mass spectrometry (MS)-based proteomics such as SRM and MRM is used for the detection and quantification of proteins in complex samples such as biological fluids or cell lysates¹. Rapid adoption has resulted from the accessible dynamic range of protein expression levels over five orders of magnitude, with detection limits as low as 50 copies of a protein per cell².

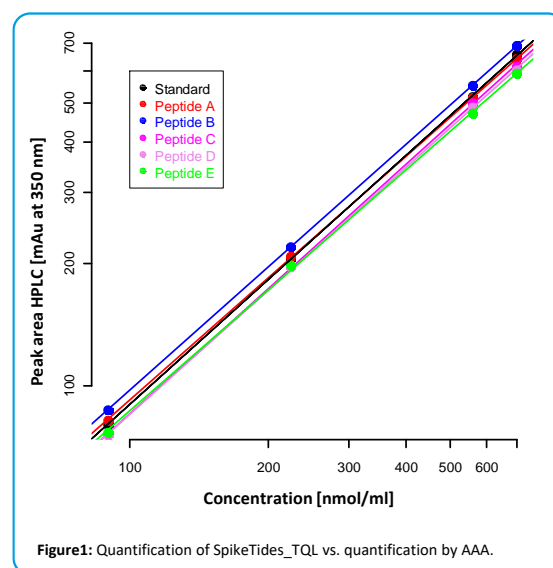
Proteotypic peptides are generated by protease-mediated digest of a protein. In SRM and MRM assays, distinctive proteotypic peptides from a protein are used for the identification of specific precursor- and fragment-ion masses in a mass spectrometer. Based on this, combined chromatographic and MS assays for single peptides and—by inference—for proteins are established. The optimized parameters can be used for the routine identification and quantification of the corresponding protein.

A growing number of SRM parameters are available to the scientific community at databases (e.g. www.srmatlas.org). However, the availability of peptides for assay development and quantification is still a bottleneck. The full potential of targeted proteomics requires a reliable source of inexpensive and quantified peptides. High-throughput SPOT synthesis is a cost-effective way for the production of huge numbers of peptides. With this method, thousands of proteotypic peptides and their heavily labeled counterparts can be produced within days at a fraction of costs of classical synthesis. To drive absolute peptide quantification to higher throughput and cost effectiveness, we have developed a quantification method that is based on a proteolytically cleavable quantification tag with unique spectrophotometric properties.

Peptide Synthesis & Quantification

SpikeTides™ are usually synthesized via SPOT synthesis^{3,4}. The SPOT method is characterized by a stepwise, Fmoc-based high throughput solid-phase synthesis on cellulose membranes. Post-translational modifications, incorporation of heavily labeled amino acids, and conjugations are possible. After synthesis, the peptides are cleaved off the membrane, transferred into ready-to-use 96- or 384-well plates and freeze-dried. At JPT, this procedure yields up to 50,000 individual peptides per week.

SpikeTides™ are rapidly and inexpensively quantified using a unique quantification tag. The tag is proteolytically labile and has UV-absorption properties that differ from those of the peptide, allowing quantification via HPLC in comparison to a standard. **Figure 1** shows calibration curves for tagged peptides and the standard. The quantification is linear over the tested concentration range (100–700 nmol/ml) and independent of peptide sequence. Overall error (SD) for quantification is 5.4%.



Principle of SRM or MRM Assays

SRM and MRM are increasingly used in targeted proteomics experiments. Initially, putative proteotypic peptide sequences from proteins of interest are selected (e.g., from PeptideAtlas (www.peptideatlas.org) or PRIDE (www.ebi.ac.uk/pride/)), or predicted. Candidate peptides are synthesized and then injected into an HPLC system coupled to a triple quadrupole mass spectrometer. Predominant peptide fragments are chosen for each peptide by selection of the peptide ion, fragmentation pattern, and selection of an indicative fragment ion. The MS parameters can be optimized to maximize the sensitivity of the assay. After validation, characteristic assay parameters are sufficient to repeat the assay and have to be established only once for a specific type of mass spectrometer and fragmentation method. Assay development can be carried out in a multiplexed fashion using over 100 peptides in a single run. For a comprehensive description, see Picotti *et al.*⁵

Applications

JPT has developed a product class SpikeTides™ that addresses all peptide needs of targeted MS-based proteomics (**Table 1**).

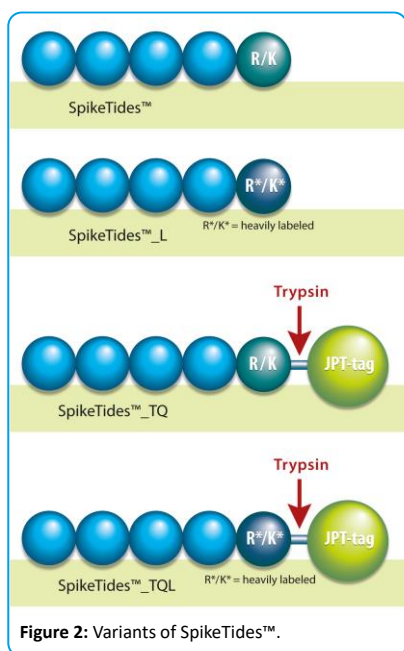
SpikeTides™ for development of SRM or MRM assays: SpikeTides™ for evaluation of their suitability for and setup of SRM/MRM assays are unlabeled and not quantified.

Heavily labeled SpikeTides_L for relative quantification: Uniformly ¹³C- and ¹⁵N-labeled arginine or lysine is used at the C terminus of SpikeTides_L. These amino acids are selected, since all proteotypic peptides that result from tryptic digestion of proteins contain C-terminal arginine or lysine. After the

SpikeTides™ Proteotypic Peptides

SpikeTides_L are spiked into the sample, two sets of fragment ions can be detected: heavy-isotope labeled (from SpikeTide_L, mass difference +8 Lys, +10 Arg) and non-labeled (from digested protein). Spiking in SpikeTides_L into different biological samples permits the relative quantification of proteins from sample to sample.

Quantified unlabeled or heavy-isotope-labeled SpikeTides™ for absolute quantification: SpikeTides_TQ (unlabeled) and SpikeTides_TQL (heavy-isotope labeled) are peptides that are quantified using a covalently bound UV-active quantification tag that is cleaved by tryptic digests in standard SRM or MRM workflows. The defined concentration of the SpikeTide_TQ or SpikeTide_TQL allows the absolute quantification of protein in a sample. The tag is designed to be of low molecular weight and hydrophilic, minimizing interference in HPLC separation and MS detection.



Examples of Use

Efficient high-throughput synthesis makes SpikeTides™ an ideal tool for the establishment of assays to measure the abundance of many proteins in a wide variety of biological samples. This has been demonstrated in *S. cerevisiae*, in which the detection limit to measure low-abundant proteins by SRM was less than 50 protein copies per cell². Furthermore, the development of SRM assays for proteome-wide detection of all kinases and phosphatases in yeast using SpikeTides™ has been described⁵. SpikeTides™ are successfully applied in a large scale project aiming at a complete map of the human proteome by MS, initiated by the Institute for Systems Biology (Seattle) and the Swiss Federal Institute of Technology (Zürich). Both, global and targeted approaches for biomarker discovery, as well as deciphering biological pathways with medical focus, benefit from SpikeTides™. Recently, SpikeTides™ were used to validate MRM assays to detect biomarkers in the feces of patients with colorectal cancer⁶. Furthermore, Hewel *et al.* set up a flexible, rapid and cost-effective assay system for the detection and quantification of low-abundance components in signaling pathways of human embryonic stem-cell populations⁷. Here, quantified labeled SpikeTides_TQL were used for the first combined quantification of key nodes in a regulatory pathway by MS-based proteomics.

Conclusion

SpikeTides™ are cost-effective peptides that allow the full utilization of MS-based proteomics by opening new dimensions in sensitivity and almost unlimited coverage through entire proteomes. They provide access to high-speed assay development and relative protein quantification and use a new approach to absolutely quantify peptides, thus eliminating laborious and expensive peptide purification and amino acid analysis (AAA) for peptide quantification. Additionally, the flexibility of chemical synthesis of SpikeTides™ permits monitoring of cellular regulation by incorporation of post-translational modifications.

References

1. Doerr, A. Targeted Proteomics. *Nat. Methods* (2010)
2. Picotti, P., Bodenmiller, B., Mueller, L.N., Domon, B., & Aebersold, R. Full dynamic range proteome analysis of *S. cerevisiae* by targeted proteomics. *Cell* (2009)
3. Wenschuh, H. et al. Coherent membrane supports for parallel microsynthesis and screening of bioactive peptides. *Biopolymers* (2000)
4. Hilpert, K., Winkler, D.F. & Hancock, R.E. Peptide arrays on cellulose support: SPOT synthesis, a time and cost efficient method for synthesis of large numbers of peptides in a parallel and addressable fashion. *Nat. Protoc.* (2007)
5. Picotti, P. et al. High-throughput generation of selected reaction-monitoring assays for proteins and proteomes. *Nat. Methods* (2010)
6. Ang, C.S., Nice, E.C. Targeted in-gel MRM: a hypothesis driven approach for colorectal cancer biomarker discovery in human feces. *J. Proteome Res.* (2010)
7. Hewel, J.A. et al. Synthetic peptide arrays for pathway-level protein monitoring by liquid chromatography-tandem mass spectrometry. *Mol. Cell. Proteomics* (2010)

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The Company

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