SpikeMix™ Peptides – A Novel Approach for Low Cost Peptide Pools in MS-based Proteomics

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Introduction

Recently, SpikeTides™ were reported as small-scale, inexpensive, heavy or light and/or absolutely quantified peptides e.g. for SRM/MRM (1) (Table 1).
Here we present an advanced procedure for the preparation of synthetic peptide pools with increased efficacy and throughput.

Development of SRM or MRM assays	
SpikeTides™ Small scale, unpurified proteotypic peptides (>50nmol)	proteotypic peptide R/K
Relative quantification	
SpikeTides_L SpikeTides™ with heavily labeled C-terminal lysine or arginine (Arg M + 10 or Lys M + 8)	proteotypic peptide R*/K*
Absolute quantification	
SpikeTides_TQ/SpikeTides_TQL SpikeTides™ with unlabeled (TQ) or heavily labeled (TQL) C-terminal lysine or arginine and absolutely quantified using a proprietary Quanti-Tag. Proteotypic peptides are released from tag by tryptic digestion. Aliquots of 5 x 1 nmol target peptide are delivered.	JPT-tag, Trypsin proteotypic peptide R#/K#

* residue uniformly ^{13}C and ^{15}N labeled
\# residue optionally uniformly ^{13}C and ^{15}N labeled

Table 1: Variants of SpikeTides™.

Methods

Peptides were prepared by SPOT™ synthesis (2). In contrast to the standard methodology, two major parameters were changed (Figure 1):

- The density of the spots on the membrane was increased by a factor of three to four;
- After synthesis, the peptides were simultaneously cleaved off the membrane in a one-pot procedure, yielding all desired peptides in a single peptide pool.

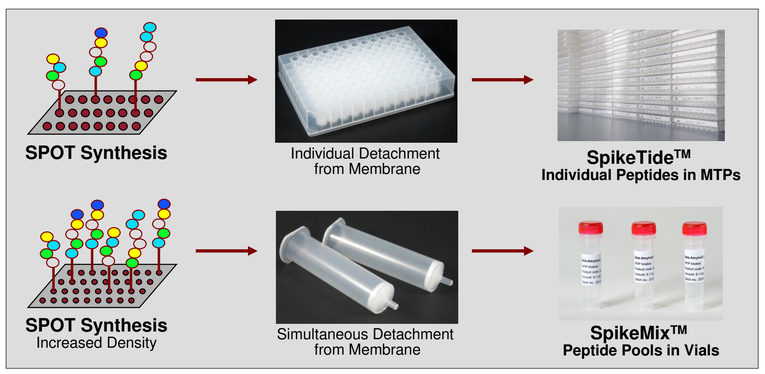

Figure 1: Comparison of the SpikeTide™ and the new SpikeMix™ technology.

The resulting peptide mixtures were analyzed via LC-MS/MS on an LTQ Orbitrap XL (CID fragment spectra) and on an Orbitrap Velos mass spectrometer (HCD fragment spectra) followed by Mascot database search for presence of assembled peptides.

Methodical Results

Synthesis Efficacy

Compared to the individual synthesis and handling of peptides followed by a laborious pooling process (SpikeTides™ approach) the newly developed SpikeMix™ method is extremely cost and time-saving. Overall, the approach reduces the synthetic effort per peptide by a factor of 2 to 3 depending on the number of peptides.

The Human Proteome in ProteomicsDB

ProteomicsDB is a new database which contains high-quality shotgun proteomics data for the whole human proteome (3, 4). To maximize data quality, reference standards to support weak peptide identifications in the database were needed. As a proof of concept study for the new technique approx. 4000 peptides distributed to 4 pools were synthesized using the SpikeMix™ technology (Figure 2). LC-MS analysis revealed that 91% of the peptides could be successfully identified (Figure 3A). Figure 3B shows an example spectrum for a peptide where the detection of an uncertain protein was validated by the peptide reference standard.

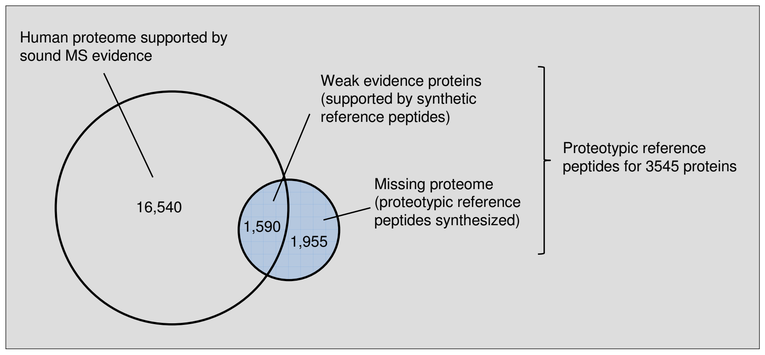

Figure 2: Selection of SpikeMix™ peptides synthesized for ProteomicsDB.

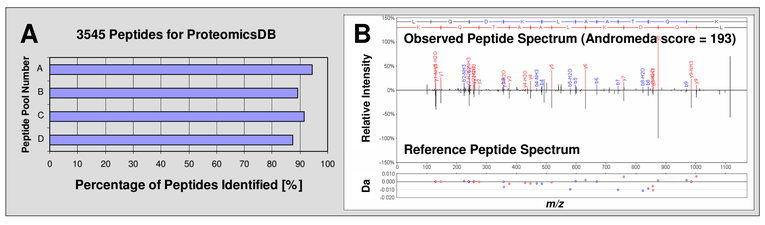

Figure 3: A) Results for the synthesis of 3545 SpikeMix™ peptides for ProteomicsDB (4 pools of 800-1000 peptides each). B) Exemplary spectrum for one of the peptides.

After confirming sufficient peptide qualities for MS-based proteomics, the SpikeMix™ was applied to generate several application examples as follows.

Application Examples

Application 1: Cytokine Pool

Cytokines are important regulators of immune processes. We prepared 461 proteotypic peptides that originate from 219 different human cytokines (3). After synthesis, more than 95 % of the peptides were successfully identified (Fig. 4). Based on these results the collection of peptides allows the simultaneous detection and relative quantification of more than 200 cytokines in biological samples.

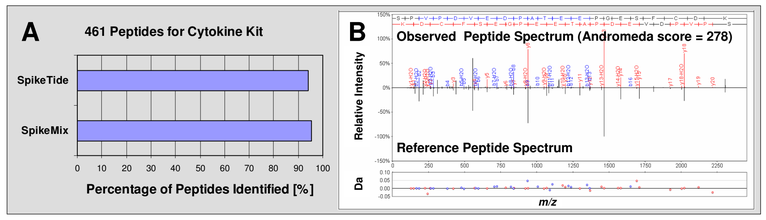

Figure 4: A) Results for the SpikeMix™ synthesis of 461 Cytokine peptides. B) Fragmentation pattern for one of the Interleukin-32 coding peptides.

Application 2: TAA (Tumor-associated antigen) Pool

A pool of 252 stable isotope labeled proteotypic peptides representative for 61 dominant human tumor associated antigens for direct usage in mass-spectrometry based proteomics (MRM) was prepared.

Application 3: ABRF (cross-species standard) Pool

A pool of 1000 stable isotope labeled proteotypic peptides (SIL) was synthesized. The peptides - derived from conserved human, mouse and rat proteins - are used as standards across the three species (5).

The new SpikeMix™ method provides light & heavy peptide pools with unprecedented efficiency. The concept is applicable to any peptide library including PTM's.

References

(1) (a) Schnatbaum, K., et al. SpikeTides - proteotypic peptides for large-scale MS-based proteomics. Non-peer-reviewed application note in *Nature Methods* **2011**, 8; (b) Picotti, P., et al. High-throughput generation of selected reaction-monitoring assays for proteins and proteomes. *Nature Methods* **2010**, *7*, 43-46.
(2) Wenschuh, H., et al. Coherent membrane supports for parallel microsynthesis and screening of bioactive peptides. *Biopolymers* **2000**, *55*, 188-206.
(3) Wilhelm, M., et al. Mass-spectrometry-based draft of the human proteome. *Nature* **2014**, *509*, 582-587.
(4) https://www.proteomicsdb.org.
(5) http://www.abrf.org/ResearchGroups/ProteomicsStandardsResearchGroup/Activities/ABRF2014_sPRGtalk.pdf

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