New methods for fast and effective synthesis of stable isotope labeled (SIL) PTM peptides

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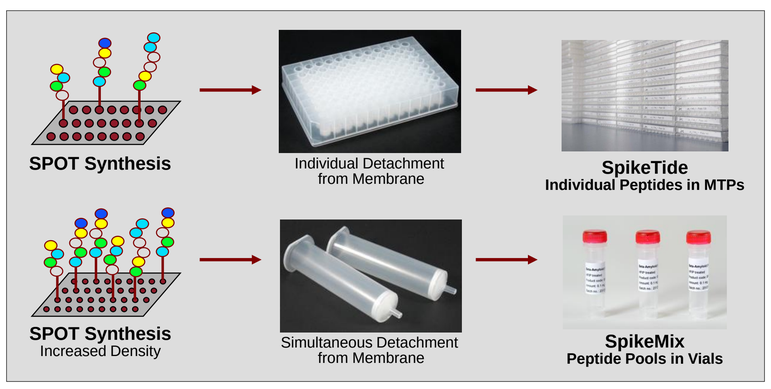
Introduction

Stable isotope labeled (SIL) or unlabeled reference peptides are pivotal for targeted proteomics and increasingly used in immunopeptidomics. Therefore, methods for the fast and efficient synthesis of such peptides are of great value. With respect to immunopeptidomics, it becomes increasingly clear that posttranslational modifications (PTMs) are relevant determinants of the antigenic landscape of the immunopeptidome,[1] thus making the rapid synthesis of PTM-modified SIL peptides an urgent need.

Here we present a chemical synthesis method enabling fast and flexible access to the above peptides currently not economically available in high numbers by standard peptide synthesis approaches.

Methods and Results

More than 1.3 million individual peptides have previously been synthesized in the course of the ProteomeTools project[2] using the SpikeMix technology, which is a very fast and efficient synthesis method based on the Spot synthesis concept (Scheme 1). The peptides covered the complete human proteome[2] and 300.000 non-tryptic HLA Class I and HLA Class II peptides[3].



Scheme 1: Comparison of the SpikeTide and the SpikeMix technology.

Additionally, various sets of PTM peptides were synthesized in light or heavy isotope labelled form (the SpikeMix technology is able to provide both equally well) and analyzed by LC-MS/MS or LC-MS.

The PTMs were incorporated by using respective pre-synthesized and suitably protected modified amino acid building blocks. Analysis by LC-MS/MS was done on a Thermo Orbitrap Fusion Lumos instrument, analysis by LC-MS on an Agilent LC/MSD Quad SL system.

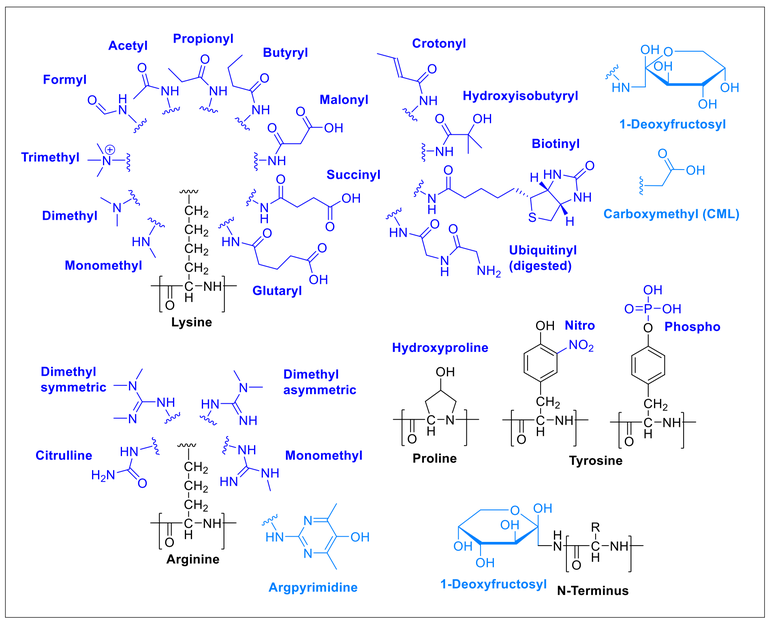


Figure 1: Overview of PTMs included in the study.

Peptides with a defined PTM (Figure 1, dark blue) were synthesized in sets of more than 100 peptides each and analyzed by LC-MS/MS.[4]

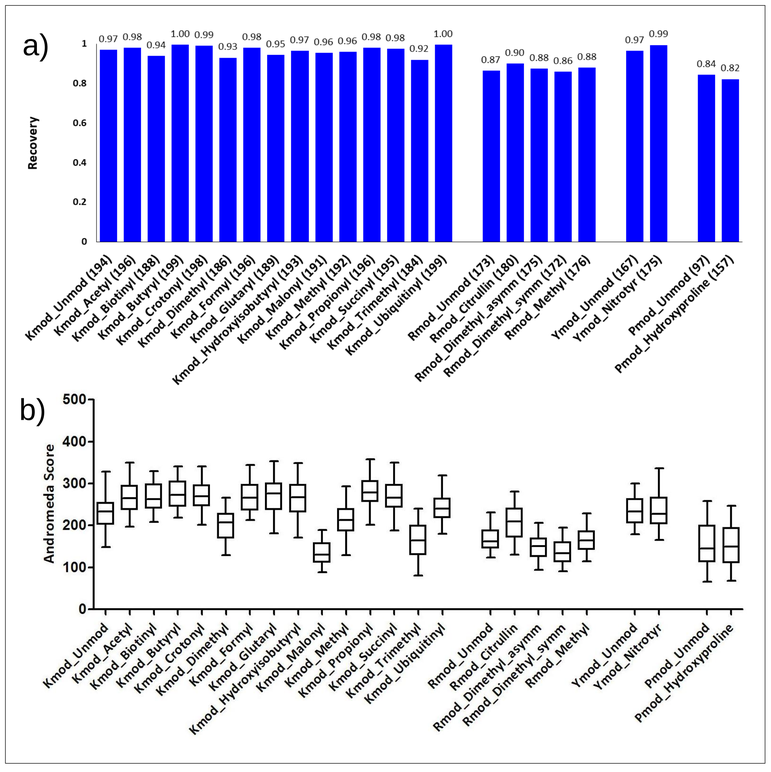


Figure 2: a) Recovery (proportion of successfully detected peptides) for PTM modified peptides. The number of synthesized peptides is shown in brackets below the bars. b) Determined MaxQuant Andromeda scores for a final peptide set of 100 selected peptides for each PTM.

The recovery for the synthesized peptides was very high (Figure 2a). Among frequently considered PTMs such as Lys(Ac) and Lys(GG) more rarely analyzed PTMs like Lys(Propionyl), Lys(Crotonyl) and Lys(Hydroxyisobutyryl) were addressed. Peptides were aliquoted in sets of 100 peptides per PTM and re-analyzed to confirm high Andromeda scores (Figure 2b).

In addition to the above mentioned PTMs we were able to extend the synthesis method to selected advanced glycation end products (AGEs) (Figure 1, light blue). The following was done to proof this: 51 Peptides covering all lysine and arginine residues as well as the N-termini of hemoglobin alpha and beta were synthesized in PTM modified form: N-Termini and lysines were modified by 1-deoxyfructosyl, lysines by carboxymethyl, and arginines by argpyrimidine. LC-MS analysis showed that recoveries were very high (Figure 3), thus underlining that the synthesis method is very effective even for complex PTMs.

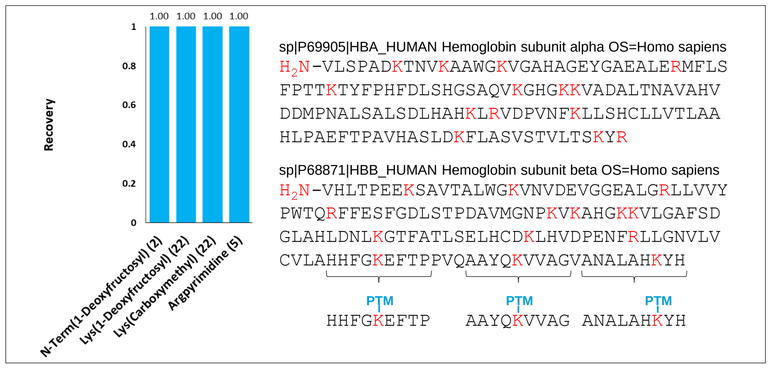


Figure 3: Recovery (proportion of successfully detected peptides) for 51 PTM peptides from hemoglobin. The PTM modified positions are marked in red. The 9-meric peptides carried the PTM at the N-terminus or as far as possible in the middle of the sequence.

Conclusion

A fast and effective peptide synthesis method has supported targeted proteomics with reference standards, including more than 20 different PTMs.

References

[1] Kacen, A., et al. *Nat. Biotechnol.* **2023**, *41(2)*, 239-251.
[2] Zolg, D. P. et al. *Nat. Methods* **2017**, *14(3)*, 259-262.
[3] Zolg, D. P. et al. *Nat. Commun.* **2021**, *12(1)*, 3346.
[4] Zolg, D. P. et al. *Mol. Cell. Proteomics* **2018**, *17(9)*,1850-1863.

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