**Introduction**

Peptide Synthesis is used for many purposes: 1) to generate large peptide libraries, 2) for affinity targets of binding assays and epitope mapping and 3) for highly pure standards for mass spectrometry.

Nearly all peptide synthesis today is based on the chemistries for solid phase peptide synthesis (SPPS) from Merrifield (1). Another common way of doing SPPS is using membranes to generate peptide SPOTs (2). However, the latter is generating peptides often in less purity and low yield. Nevertheless, it has been recently applied to generate mass spectrometry standards on a large scale (3) while it was also used for peptide-affinity screens or epitope mapping (4). Here we show an improved approach for rapid peptide synthesis of hundreds of peptides a day.

**Method**

- Manual or robotic manipulation (pipetting).
- All steps were done with the same chromatographic tip and polypropylene well plates (either 96, 384 or 1536).
- Using AA-functionalized tips the following AA building blocks can be added through FMOC chemistry (deprotection of FMOC group and coupling cycles).
- If desired synthetic peptides can be eluted from the media.
- LC-MS results can be readily performed on eluted peptides.
- For some peptides a purity of up to 98% can be obtained with an amount of 0.1-10 nmol.
- Minimal solvent consumption (i.e. <2 L waste for 384 15-mer peptides).

**Configuration for Manual or Automated Peptide Synthesis**

- Pipette tip packed with chromatographic media for synthesis = PepTip™
- Peptide sequences can be synthesized by AA-1 activation.
- Peptide is coupled to the C-terminus of the previous amino acid.
- Side group deprotection can be performed after coupling cycles.
- Peptide synthesis is terminated when the target sequence is obtained.
- Peptides are washed and precipitated on the tip.
- Peptides are eluted from the tip.
- Peptides are lyophilized and stored at -20°C.

**References**


**High-Throughput Peptide Synthesis using BRAVO Liquid Handler**

The automated peptide synthesis has been already established for decades. Also the high-throughput synthesis of peptides on cellulose membrane has been widely used for generating entire peptide libraries and has been used in peptide affinity screening experiments, e.g. epitope mapping. However, slower speed of parallel synthesis is limiting synthesis productivity. With common liquid handling robots it is now possible to handle minute amounts of <1 μL accurately. This opens the possibility to do synthesis on small scale but in unprecedented parallel action and at high-speeds. Below we show the first example of the feasibility to obtain soluble peptides as well as peptides linked to solid support with a rapid synthesis protocol using a commercial liquid handling platform.

**Absolute Quantification**

Accurate quantification of peptides is a key aspect and long term goal for proteomics. Since peptides in mass spectrometry undergo different ionization efficiencies the generation of calibration curves is required. Here we show in an example of a short peptide VVYVK that it is feasible to create standard curves for peptides obtained through this synthesis protocol. However, such experiments need to be shown on large scale for many peptides in parallel.

**Affinity Purification**

Screen purified proteins: Purify cell lysates:

On-tip digestion

Quantitative LC-MRM analysis of purified proteins bound to peptide libraries.

LC-MS analysis of proteins and protein identification

**Conclusions**

- The chromatographic tips allow rapid, reproducible and parallel peptide synthesis, when larger amounts are needed as standards for mass spectrometry or to spot many microarrays.
- We created an automated and easy to use liquid handling protocol for manual pipettors (small scale) or robots (large scale).
- Peptides obtained with up to 98% purity and approximate amount of 10 nmol.

**Synthesis-in-a-Tip: An optimized protocol for high-throughput peptide synthesis using tips packed with chromatographic media.**

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