Massive Parallelization

PEC in a 96-well filter plate

The absence of by-products and other impurities in peptide libraries, synthesized via SPPS, is vital for reliable assay results. However, especially in screenings (N > 24), potentially low-purity crude peptides are often used because parallel purification is not feasible. We overcome this bottleneck by customization of our PEC technology to a 96 well-plate format.

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1. Introduction

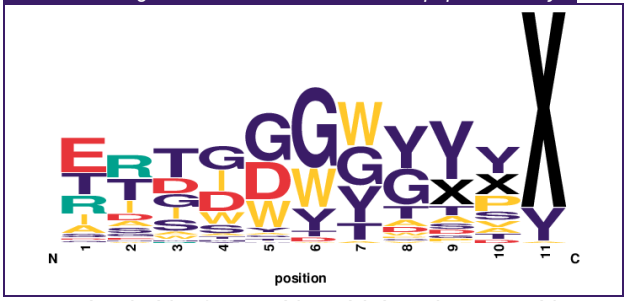
During a peptide drug development process and especially in the lead-optimization step, screening for a large number of different peptides is essential. Modern parallel solid-phase peptide synthesis (SPPS) following the Fmoc-strategy, for instance, enables the automated assembly of multiple peptides in short times. However, by-products such as truncations or deletion sequences typically form during the synthesis due to incomplete couplings. These impurities can be removed by preparative reversed-phase high-pressure liquid chromatography (RP-HPLC). However, RP-HPLC is limited in throughput, as only one peptide per column can be purified. Consequently, current screenings mostly rely on crude peptides, which increases the risk of false-positive or false-negative results, impairing the further development of peptidic drugs.[1]

In this case study, we present a proof-of-principle, using a well-plate format and a vacuum manifold for PEC processing that allowed us to purify 96 peptides in a ten µmol scale simultaneously.

2. Method

Synthesis. Two sets of 96 peptides were synthesized in parallel in a 10 µmol scale on Ramage-Amide-DP-Resin in 96-well plates (1 x 45 min with a 5-fold excess of aa), followed by routine capping (2 M Ac2O, 2 M pyridine in DMF 1x5 min). This capping enabled selective coupling of the reductively cleavable linker RC+ (4 eq.) to the full-length peptide as the last building block in DMF using DIPEA (6 eq.) and Oxyma (4 eq.) for 2 h. Sequences were kindly provided by Steven Cobb, Department of Chemistry, Durham University, UK. TFA cleavage was performed for 2h using TFA/H_2O/EDT/PhSMe/TIS (83:5:5:5:2) (0.5 mL cleavage cocktail per 10 µmol peptide). The crude peptides were collected, precipitated, centrifuged and dried in a 96-well plate Table 1 shows common attributes of the selected peptide set.

Table 1: Weblogo of the used residues for the peptide library



X = placeholder for peptides with less than 11 residues

PEC purification. For parallel purification of the library, the PEC procedure was adapted to the 96 well plate format. Hence, after dissolution of the crude linker-modified peptides, all further steps were performed in a fritted 96-well filter plate with the help of a vacuum manifold, which allows for a seamless workflow from immobilization to final release. The purified peptides were collected in a deep well plate and directly lyophilized. Only the peptides of the first synthesis plate were purified using the PEC technology and lyophilized. The peptides of the second plate were cleaved, precipitated, dissolved in H_2O/MeCN (7:3) + 0.1% TFA, analyzed, and lyophilized without further purification to determine crude purities. Analysis. UPLC-UV and mass spectra were recorded with an analytical Acquity H-Class UPLC-ESI-MS system from Waters on a C-18 column (1.7 µm, 2.1 x 500 mm). As the mobile phase, mixtures of water (A) and MeCN (B) with 0.1% TFA were used.

Recoveries were calculated using an internal standard with a known extinction coefficient at 210 nm (0.1 mg/mL Fmoc-L-Glutamic acid-(OtBu) monohydrate solution). The addition of extinction coefficients (ε_{abs}) of the amino acids at 214 nm allowed us to calculate the ε_{abs} of the target sample.[2]

3. Discussion

The mean purity from 96 peptides could be increased by 19% using PEC technology (Table 2). No truncated sequences could be detected. Only one peptide showed a purity below 70%. This is a major improvement compared to the crude plate, where only 18 peptides showed purities above 80%. The overall recovery of peptides was 48%.

Table 2: Mean results for crude and PEC purified peptides.

	$N_{eptides}$	mean purity / %	mean yield / µmol
crude	96	68%	4.4 µmol
purified	96	87%	2.1 µmol

Figure 1: UPLC-UV/vis-chromatograms of crude and PEC-purified SCP12 and SCP31 at 210 nm

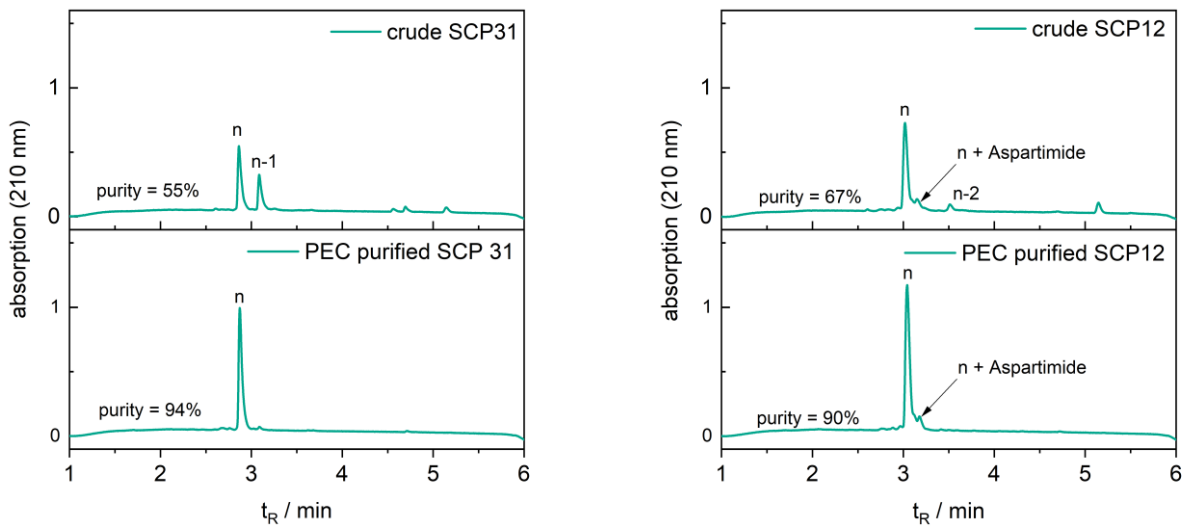


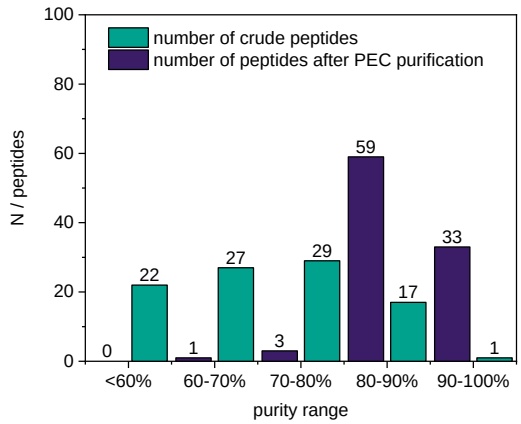
Figure 1 shows the crude and PEC-purified chromatograms for a more detailed view of two representative peptides.

The example of SCP31 (Figure 1, left) highlights the core strength of PEC: the efficient removal of truncated sequences. This feature is essential in unoptimized syntheses, such as in the preparation of peptide libraries.

Impurities caused by aspartimide formation were dominant in this set as 76 of 96 peptides contain an Asp-Gly-segment in their sequences. An example is shown in Figure 1 (right). On average, impurities caused by aspartimide formation account for 7%.

Even though PEC could not remove these impurities at this point, these results showcase the impressive potential: Appropriate measures, such as using Asp(Dmb/Hmb)Gly building block, would enable a mean purity above the 90% level for the parallel purification of 96 peptides in a filter plate format.

Figure 2: Crude and PEC purity analysis of 96 peptides



4. Results at a glance

- access massive parallel purification of peptides with PEC in a 96-well filter plate format
- use cost-efficient purified libraries to improve your assay reliability
- shorten the time and cost to prepare purified libraries with PEC

5. References

[1] Currier J. R.; et al. *Clinical and Vaccine Immunology* 2008, 15, 267-276.

[2] Kuipers B. J. H.; Gruppen H. *J. Agr. Food Chem.* 2007, *55*, 5445-5451.

About Belyntic

Belyntic GmbH is a chemistry-for-healthcare enterprise focusing on the purification of biopolymers, especially peptides. Belyntic offers the world's first broadly applicable peptide purification kits as well as development and implementation services of its proprietary Peptide Easy Clean (PEC) technology.

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