The new dimension of purity

Peptides vs Virus

The adaption of the PEC technology to a 96 well-plate format gives fast access to purified peptide libraries. PEC removes most peptidic impurities that may cause false-positive signals and improves subsequent screening or the reliability of validation assays. These "PEC-grade" peptide libraries show excellent performance in validation studies of SARS-CoV-2 epitopes, likely to elicit a T cell response. This case study highlights the parallel purification's potential during the development of in-silico designed peptide drugs or vaccines.

Stephan Lüdtke[1], Robert Zitterbart[1], Natalio Garbi[2]

[1]Belyntic GmbH, Berlin, Germany

[2]Institute of Experimental Immunology, University of Bonn, Bonn, Germany

1. Introduction

The COVID-19 pandemic has unleashed an unprecedented global collaboration force to find a vaccine. Despite a rich pipeline of promising projects, candidates that target T-cell responses and cellular immunity remain rare. However, this immune response type is vital to engage long-term and robust protection against viral infections such as SARS-CoV-2.[1]

T cell activation assays with rationally selected peptide epitopes from the SARS-CoV-2 proteome help identifying immunogenic vaccine candidates. The fast supply of peptide libraries covering a range of, for example, in-silico predicted candidates is vital to improve the experimental throughput and chance to capture ideal target sequences in a short time.

However, the T cell assay outcome is critically affected by the quality of the peptides used: unpurified peptide libraries contain specimens contaminated with truncation or deletion sequences which can cause false-positive results. Such impurities often coelute and cannot be removed with conventional chromatography.[2] A chemoselective isolation strategy allowing for parallel processing to increase peptide purity of a whole library is, therefore, essential before performing relevant T cell activation assays.

In this case study, a peptide library of 96 SARS-CoV-2 epitopes with free C-terminal acid was purified using PEC in plate format, and employed to activate $CD8^+$ T cells from convalescent COVID-19 patients. For peptide libraries, we introduce the "PEC-grade" label, defined by the absence contaminants such as deletions, truncations or cross-contaminations, that may cause false-positive assay results.

2. Method

Synthesis. The 96 SARS-CoV-2 MHC class I peptide-epitopes were selected by Natalio Garbi. They covered all 20 canonical amino acids. Fmoc-synthesis was performed in parallel (10 µmol scale) on preloaded TCP resins in a 96-well reaction plate to generate the C-terminal acid (1 x 45 min coupling with a 5-fold excess of aa). Capping, following our routine protocol (4 M Ac_2O in DMF, 4 M pyridine in DMF, 1x5 min), enabled selective coupling of the reductively cleavable PEC-Linker RC+ (4 eq.) in DMF for 4 h to the full-length peptide as the last building block using DIPEA (6 eq.) and Oxyma (6 eq.). TFA cleavage was performed for 2 h using TFA/H_2O/EDT/PhSMe/TIS (83:5:5:5:2) for methionine containing peptides and TFA/H_2O/DTT/TIS (88:4:6:2) for all other peptides (0.5 mL cleavage cocktail per 10 µmol peptide). All methionine containing peptides were treated for 15 min with EDT/TMSBr (8 µL/7 µL) to reduce oxidized methionine. The crude linker-modified peptides were collected, precipitated, centrifuged, and dried in a 96-well plate. Repetitions were synthesized in 5 mL reaction columns in a 50 µmol scale on preloaded TCP resins (1x 60 min coupling with a 3-fold excess of aa).

PEC purification. Peptides were purified in parallel in a 96-well plate format. The Belyntic vacuum manifold allowed a seamless workflow from immobilization to final release. The purified peptides were collected in a deep well plate and directly lyophilized. Peptides with oxidized ethionine were dissolved in 0.5 mL 95% TFA in water and treated with EDT/TMSBr (8 µL/7 µL) for 15 min. Repetitions were purified in cartridges.

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.

ELISpot assay of IFNγ secretion. PBMC-samples were obtained from patients recovered from COVID-19 at least one month before PBMC collection. IFN_γ secretion was quantified by ELISpot after challenge of the PBMC-samples for 24 h with nothing (-) as negative control, phytohemagglutinin (PHA) as positive control for IFN_γ secretion, peptide pools (P1 – P6) containing ~10 peptides each, provided by Belyntic, and the same peptide pools after blocking with an HLA-I inhibitor.

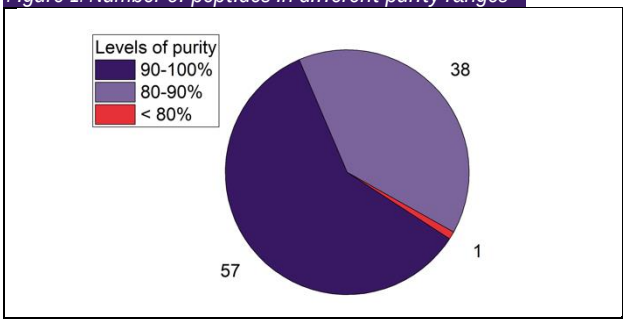
3. Results and Discussion

Library synthesis and purification. In total, we ran ten repetitions for peptides with a UV-purity below 80%. Those peptides possessed mainly hydrophobic and

aggregation-prone sequences. Synthesis on a higher scale and purification in cartridge helped to increase the purity for 9/10 peptides. Only one peptide remained below 80%.

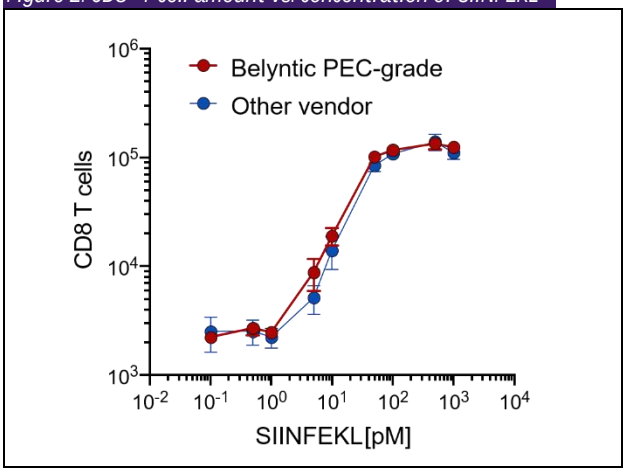
In total, 95 of 96 peptides met the PEC-grade quality requirements. On average, we determined a UV-purity of 90%, and obtained 3.8 mg purified peptide from the 10 µmol scale synthesis (Figure 1).

Figure 1: Number of peptides in different purity ranges



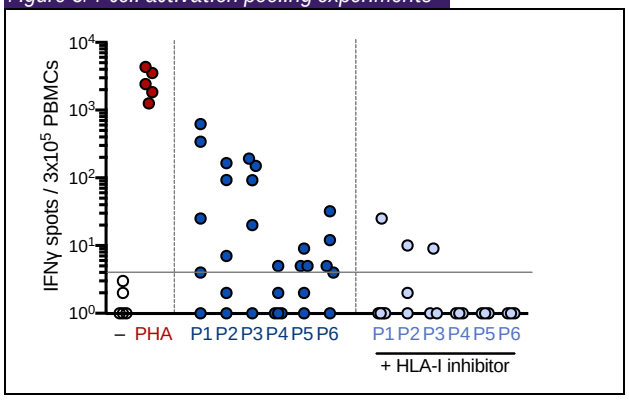
PEC-grade in ELISpot assays. We first measured OT-I CD8⁺ T cell response after 72 h treatment with SIINFEKL peptide to prove PEC-grade's suitability for T cell activation assays. For comparison, we employed a peptide provided by another vendor. Figure 2 shows the increase in CD8⁺ T cell numbers as results of cellular proliferation in response to titrated amounts of peptide. The result indicates that PEC-grade peptides induce a comparable dose-response and hence performance to conventional synthesis and purification.

Figure 2: CD8⁺ T cell amount vs. concentration of SIINFEKL



T cell activation assays. Next, the Garbi lab developed a 24 hours ELISpot-protocol to measure IFNγ secretion of PBMC-samples treated with predicted peptide epitopes from SARS-CoV-2. The employed pools contained peptides covering the most dominant HLA-types of the European population.
The experiment showed a positive response to all pools (Figure 3). P1, P2, and P3 pools contained peptides with the highest response signal, yet, these pools showed a positive reaction in some of the patients even with HLA-I inhibitor. The variation between signal intensities may result from HLA variants between patients.

Figure 3: T cell activation pooling experiments



The results show that an experimental workflow from epitope selection to epitope validation in preclinical vaccine development is feasible using PEC-grade peptides. False-positive results are unlikely since truncations are absent. In the next steps, we aim to further explore PEC-grade peptides in immune therapy, monitoring and other immunology applications.

4. Results at a glance

- Discover PEC-grade as a quality label of peptides for preclinical vaccine development
- Synthesize PEC-grade peptides in parallel with unprecedented speed using the 96-well plate format
- Employ PEC-grade peptides in T-cell activation assays for the development of, e.g., SARS-CoV-2 vaccines

5. References

[1] Nelde, A. et al. *Nat* Immunology 2020, 1-25.

[2] Currier, J. R. et al. *Clin Vaccine Immunol* 2008, *15*, 267-276.

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.

Get in touch for more information
Email: support@belyntic.com
Phone: +49 30 81041113
or visit us online at belyntic.com