An all-in-one automated peptide purification and synthesis solution

PurePep®

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Introduction

Rapid parallel production of high-quality synthetic peptide sequences by solid-phase peptide synthesis (SPPS) and subsequent purification is a challenging yet essential pursuit. Neoantigen peptides, in particular, with medium to long lengths used in cancer immunotherapies need to be manufactured rapidly and with high purity. However, conventional batch-synthesis is slow and purification with sequential high-pressure liquid chromatography (HPLC) offers low throughput, leading to extended production times and potential product loss requiring repeated synthesis. Shorter and more reliable production timelines are essential to ensure the rapid delivery of life saving personalized medicine to patients. This study presents a PurePep® solution to improve neoantigen production for cancer therapy. The integrated approach includes induction heating (IH) for rapid automated peptide synthesis followed by automated orthogonal PurePep EasyClean (PEC™) purification on the PurePep Chorus synthesizer.[1]

Methods

We chose a set of six neoantigen sequences (#1-#6) from the literature that are severely challenging to synthesize and purify (Table 1).[2] Induction-heated solid-phase peptide synthesis (IH-SPPS) at 0.1 mmol scale was performed with 17 min synthesis cycle time (incl. capping):

- Coupling: DIC/Oxyma 3 min at 90°C
- Capping: Ac_2O/Pyr. 2 min at 50°C
- Fmoc-deprotection: DMF/Pip. 1 min at 90°C
- PEC-Linker coupling on the synthesizer with Oxyma/DMF for 15 min at 60°C.
- On-board TFA cleavage at RT for 2 h (83:5:5:5:2 TFA/H_2O/PhSH/EDT/TIS)

Purification was performed using the PEC Auto Kit with the pre-configured protocol for automated peptide purification on the PurePep Chorus synthesizer.

A final HPLC polish with Knauer Semi-prep. HPLC, C18 column 15 x 2 cm was applied to obtain clinical grade peptides

Table 1. Sequences of neoantigen peptides used in this study.

ID	Sequence
#1	SNLDITPDDPRWIRAWWGGFLLCGA
#2	DWGGQHHGLREVLAAALFASCLWGA
#3	YSLDSSGNQNLYAMYQLSHFQSISVL
#4	FTLQIRGRERFEMYRELNEALELKD
#5	TLQIRGRERFEMYRELNEALELK
#6	TMLVSSLRDHFPDLPLHIHTHDTS

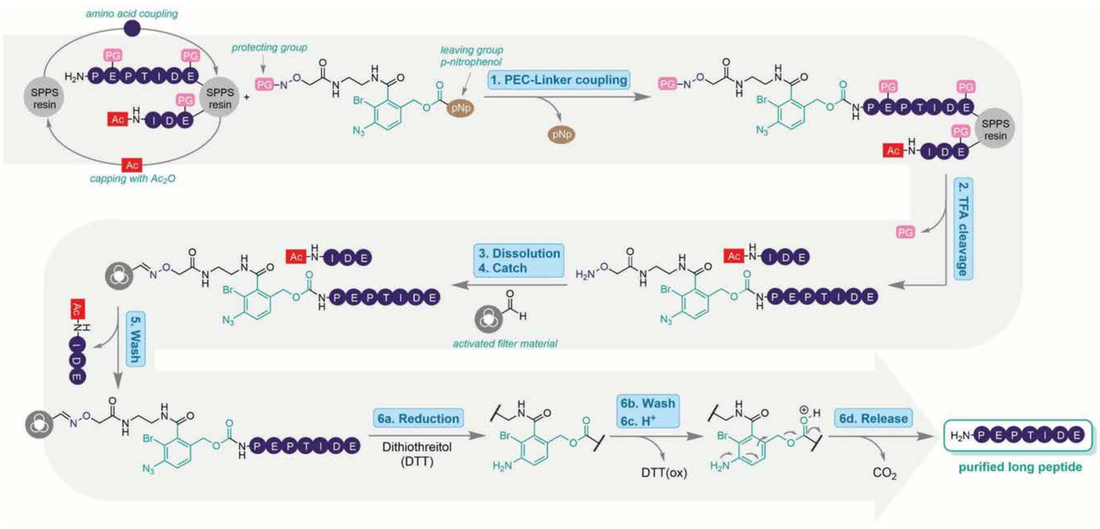

Figure 1. Chemical steps of the PEC technology used in the PEC Auto Kit, utilizing the universal linker system and its reduction-triggered safety release.

Results & Discussion

Table 2 displays the results after IH-SPPS as well as PEC purification in comparison to reported purities by Truex et al. who synthesized these peptides with the help of flow-chemistry. According to the literature, five of six peptides couldn't be delivered from an external vendor, proving their challenging profile.[2]

Table 2. Reported and gained UV-purities at 210 nm.

ID	Crude IH-SPPS	Flow-SPPS + HPLC	IH-SPPS + PEC	IH-SPPS + PEC + HPLC
#1	46%	45%	78%	94%
#2	59%	69%	80%	95%
#3	16%	53%	72%	96%
#4	60%	56%	78%	94%
#5	65%	92%	89%	94%
#6	16%	99%	74%	93%
Mean	**44%**	**69%**	**79%**	**94%**

- IH-SPPS enabled sequences that failed using batch synthesis thus providing a means to highly reliable synthesis of most difficult peptides
- Orthogonal purification with the PEC Auto Kit yielded superior PEC-grade mean purity versus flow-SPPS with subsequent HPLC using the routine protocol
- HPLC polish after PEC yielded >90% final purity for all reported difficult sequences.

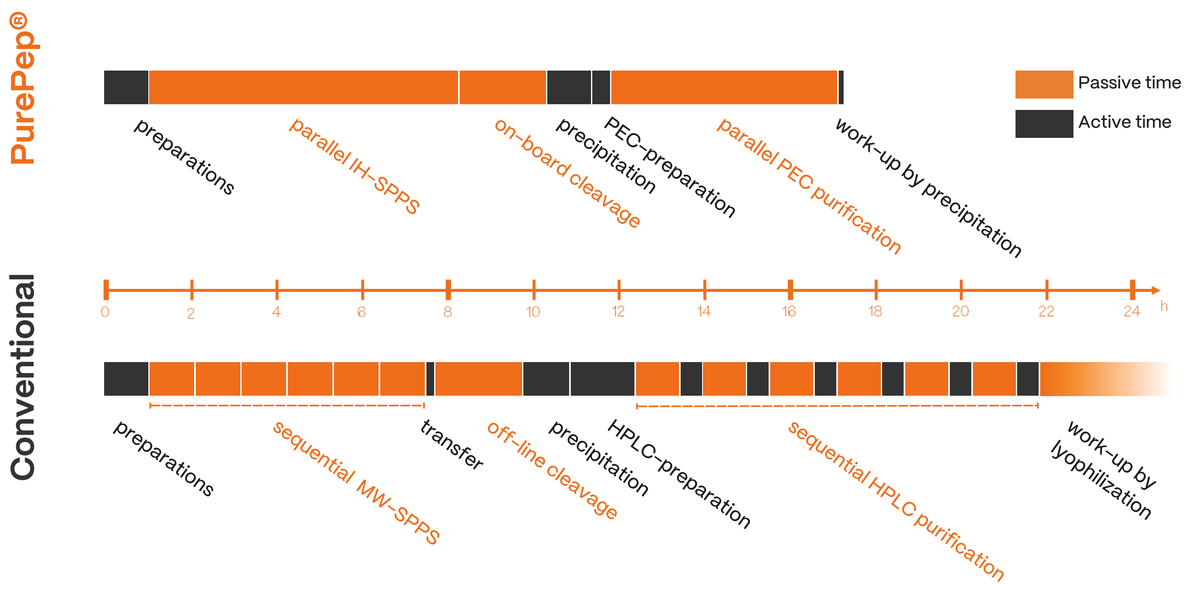

Figure 2. Time needed for parallel IH-SPPS, automated on-board cleavage and subsequent automated purification with the PEC Auto Kit for 6 peptides at 0.1 mmol scale.

- 17 min synthesis cycle time with induction heating resulted in ~7 h total time per set of 6 25-mer peptides (incl. capping)
- On-board cleavage enables hands-off workflows to obtain final product over night
- Automated purification of 6 peptides "ready to collect" in 5.2 h with only 26 minutes total hands-on time for preparation of the PEC run
- 20% faster with only one manual intervention compared to a sequential microwave synthesis and HPLC purification workflow with 7 manual interventions

PurePep Solution

PurePep® Chorus

PurePep® AutoKit

- PurePep Chorus combines IH-SPPS, automated cleavage and purification in one instrument
- The PurePep Pathway features zero cross-contamination for maximum purity
- Upgradable design for 2, 4 or 6 reaction vessels and independent induction heating and UV-monitoring
- Save reagent cost with Single-Shot™ delivery with no dead volume and zero priming

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References

[1] R. Zitterbart et al. Chem Sci 2021, 12, 2389
[2] N. L. Truex, B. Pentelute et al. Sci Rep 2020, 10, 723

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