Greener manufacturing of complex peptides with PurePep® end-to-end solutions

a collaboration with Prof. Papini from PeptLab at University of Florence

GYROS PROTEIN Technologies

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Introduction

Over the past few decades, environmental concerns have become increasingly urgent, particularly in the context of manufacturing processes involving chemicals. Traditionally, solvents such as DMF, NMP, and TFA have been used in solid-phase peptide synthesis (SPPS) despite their negative impact on the environment. Besides, Acetonitrile is heavily used for purification with conventional chromatography. However, the use of such solvents conflicts with the current trend toward green solvents.[1]

In this study, we investigated the use of PurePep® Chorus to produce two difficult peptides, Amyloid-β (1-42) and a 72-mer motif of the SARS Cov-2 virus, minimizing the use of DMF through greener DMSO/EtOAc mixtures.[2] Despite the absence of DMF during washing, yield and crude purity remained comparable to those obtained using DMF.

We further improved the sustainability of the process by implementing the orthogonal PurePep® EasyClean purification technique.[3] This catch-and-release method resulted in high quality peptides while minimizing the impact of solvents on the purification step.

Results

Synthesis with PurePep® Chorus

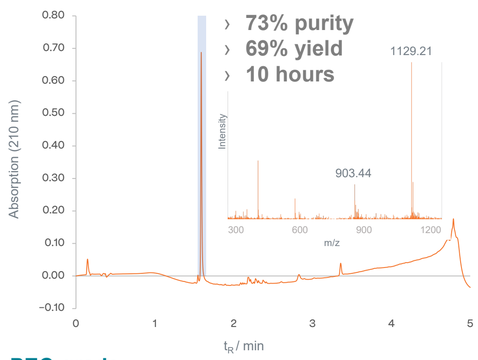
Comparison of DMF and DMSO/EtOAc mixtures for coupling

Peptide	Coupling agent	Crude UV-Purity (%) in DMF		Crude UV-Purity (%) in DMSO/EtOAc	
		Rt	50°C	Rt	50°C
Poly-Ala	DIC/ Oxyma	36	79	82	67
	HCTU/ NMM	79	78	29	85
ACP	DIC/ Oxyma	64	96	82	97
	HCTU/ NMM	86	89	85	88
G-LHRH	DIC/ Oxyma	81	88	78	76
	HCTU/ NMM	88	87	78	82

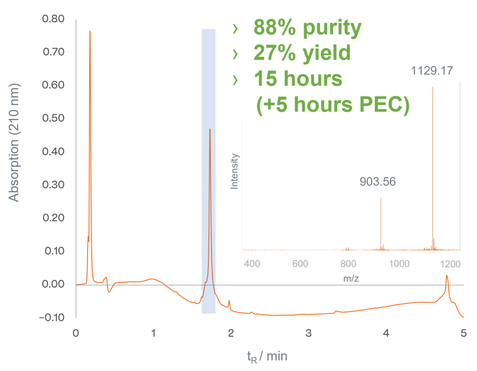
Poly-Ala: **AAAAAAAAAAK;** ACP: **VQAAIDYING;** G-LHRH: **GHWSYGLRPG**

Amyloid-β (1-42)

Crude

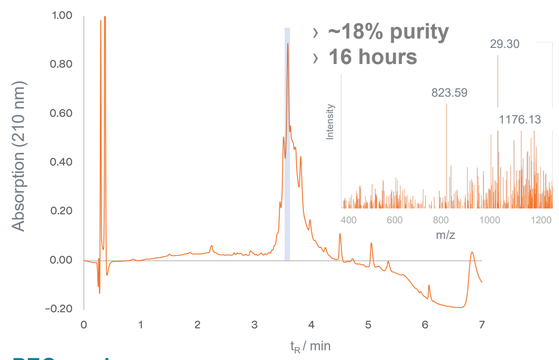


PEC-grade



72-mer SARS CoV-2 motif

Crude



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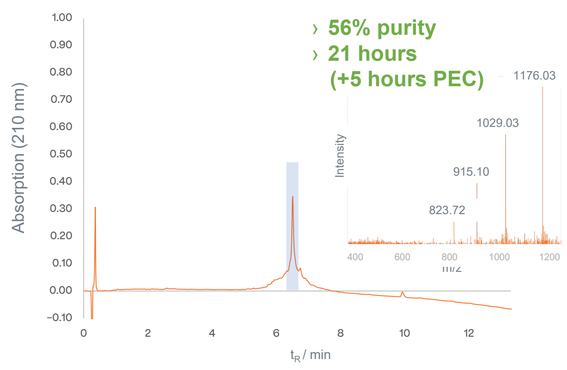
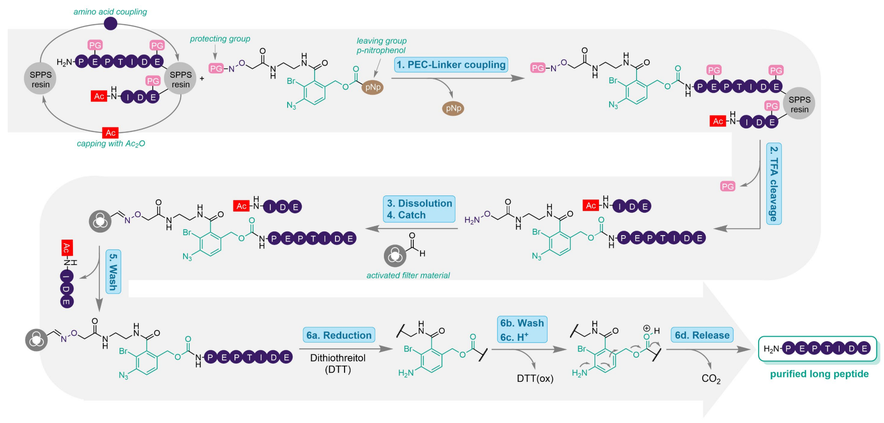
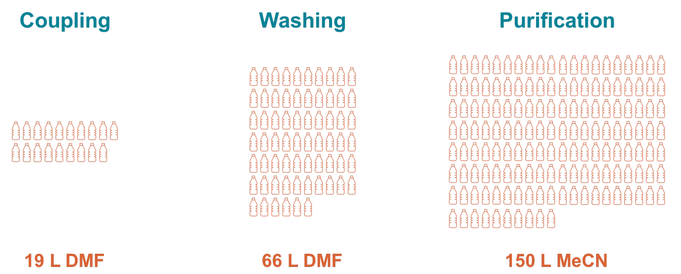


Purification with PurePep® EasyClean



Environmental impact

- Conventional solid-phase peptide synthesis (SPPS) rely on DMF for coupling and washing, purification with HPLC is mainly performed with Acetonitrile (MeCN)
- Annual solvent consumption based on 6 peptide synthesis (40 AA) per week including one subsequent HPLC purification



- The use of DMSO/EtOAc binary mixtures in conjunction with PEC purification reduces toxic solvent use significantly

Method highlights

- PurePep® Chorus was used for synthesis of both peptides
  - Amyloid-β (1-42) DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA
  - 72-mer SARS CoV-2 motif WNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGV EGFNCYFPLQSYGFQPTNGVGYQP
- Coupling was performed at 90°C and capping at 60°C using induction heating with PurePep® Chorus
  - Total AA coupling and capping time 5.7 min
- DMSO/EtOAc mixtures (8:2 v/v) were employed for washings
- PEC-Linker RC+ was installed at the end of SPPS
- Amyloid-β was treated with TFA/H_2O/EDT and precipitated in cold ether to reduce Met(ox)

Conclusions

- Rapid synthesis with induction heating on PurePep® Chorus
- Orthogonal catch-and-release purification with PurePep® EasyClean (PEC)
- No DMF required / 95% less ACN used
- Amyloid-b (1-42) with 88% purity in over night
- 72-mer long peptide with high peak purity in less than one day

References

[1] L. Ferrazzano et al. Green Chemistry **2022**, 24, p. 975-1020.
[2] D. Prat et al. Green Chemistry **2014**, 16, p. 4546–4551.
[3] R. Zitterbart et al. Chemical Sciences **2021**, 12, p. 2389-2396.

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