Chemo-Selective Catch-and-Release Purification: An Orthogonal Alternative When Chromatography Fails

PurePep®

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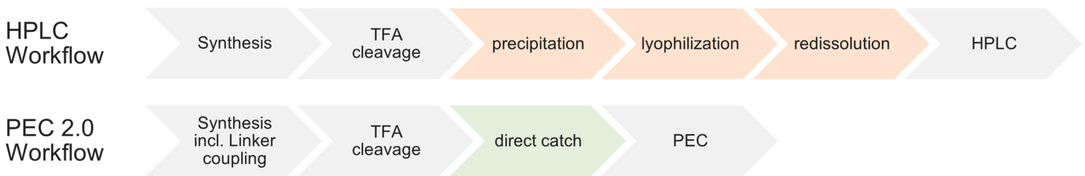
Introduction

The problem it solves: Reversed Phase High-Pressure Liquid Chromatography (RP-HPLC) fails on peptides that won't redissolve, flush through without retention, or co-elute with impurities; PurePep EasyClean (PEC™) captures peptides by chemical reactivity instead of hydrophobic interaction

What's new in 2.0: TFA-stable polymethacrylate (PMA) beads allow direct capture straight from the cleavage cocktail, eliminating ether precipitation and redissolution, and with them the oxime side products and solubility failures that plagued PEC 1.0's agarose workflow.

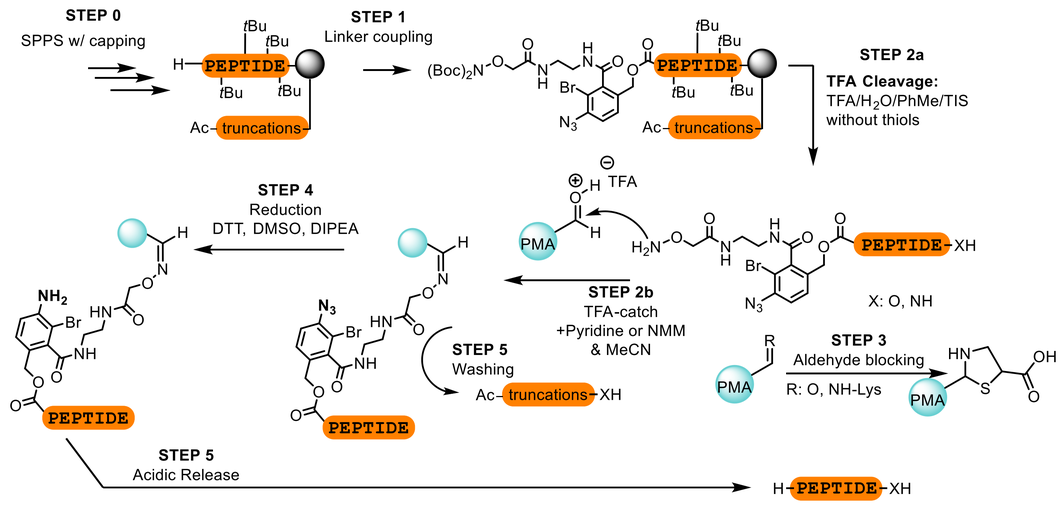
Demonstrated results: Validation across many peptides, 32 peptides in High-Throughput (including known-difficult, very hydrophilic, and very hydrophobic sequences) with mean purity jumping from 50% to 84% with up to **96 purifications/day**, and roughly 20x less solvent than HPLC.

Enabling a redissolution free workflow



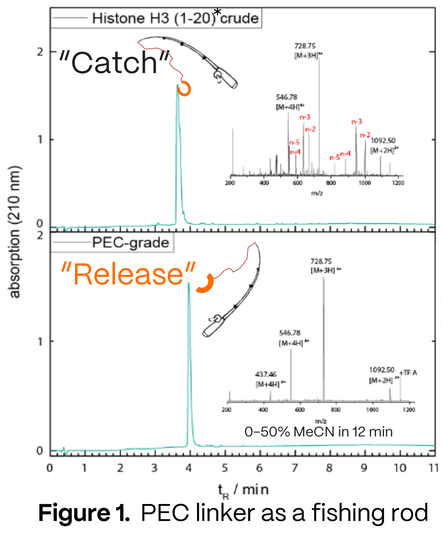
- The first generation of PEC (1.0)[1] wash using crosslinked agarose beads and relied on ether precipitation and redissolution in DMSO/citrate buffer 9:1 or neat HFIP.
- This method suffered from ketone impurities in the ether or in the solvents used for redissolution.
- Additionally, the mentioned solvents weren't always able to solubilize aggregating and hydrophobic peptides.
- To address these pitfalls, we established a TFA-catch method that uses the TFA cleavage cocktail as the medium for the catch step, thus enabling:
  - ✓ No ether precipitation, meaning no risks of unwanted oxime side products
  - ✓ Redissolution-free workflow, thus no problems with solubilizing aggregating and/or hydrophobic peptides

PurePep EasyClean (PEC2.0) mechanism



Scheme 1. Mechanism of PEC purification[2]

Benefit of Catch-And-Release purification



91% UV purity
28% peak purity
(ESI-MS)

96% UV purity
95% peak purity
(ESI-MS)

Figure 1. PEC linker as a fishing rod

- We have developed PEC to ease up peptide purification.
- The PEC-Linker RC+ is a fishing rod for peptides – installed on the N-terminus or amino side chain, fishing out the desired peptide from even the most complex mixtures!

*H3(1-20): H-ARTKQTARKSTGGKAPRKQL-NH_2, X: hydrophobic (L, I, V, W, M, F), Z: intermediate (H, S, T, P, Y, C, G, A), B: hydrophilic (R, K, E, D, N, Q)

Table 1. Comparison of HPLC, PEC1.0 and PEC 2.0 purification of peptides.

	RP-HPLC	PEC 1.0	PEC 2.0
Capture Media	C4-C18 coated silica	Agarose beads	TFA-stable PMA beads
Ether precipitation	Required	Required	Eliminated
Peptide redissolution	Required	Required	Eliminated
Ease of use	Automated; Needs development for each peptide	Multiple critical manual steps	Streamlined workflow
Robustness	Sequence-dependent	More user-dependent	Significantly more robust
Purity	Very high: Dependent on type and amount of impurities	Good purification performance	Improved purity in validation and beta tests
Yield / Recovery	0-80%	10-90%	50-90%
Hydrophilic peptides	Tendency to elute too quickly	Working well	
Hydrophobic peptides	Difficulty to solubilize and to isolate	More challenging	Working well
Aggregating peptides	Difficult to solubilize and to isolate	More challenging	Working well
Scalability	Each new scale demands a new column or device	Easily scalable	
Throughput/day	2-6 runs	16 (Starter Kits) 96 (2-days HT-Kit)	16 (Starter Kits) 96 (HT-Kit)
Solvent per peptide	500-1'000 mL	50 mL (Starter-Kit) 20 mL (HT-Kit)	30 mL (Starter-Kit) 12 mL (HT-Kit)
Applicability	Almost all peptides	Not compatible w/ N-term acetylation or azides	

Purification Results

- Purification of the peptide hormone Met-Somatostatin by PEC-cartridge (Figure 2)
- Purification of a library of 32 peptides with the PEC high-throughput kit (Figure 3)
  - ✓ 12 validation peptides (10-20 res.), including Aß 1-20, Barstar (75-90), JR10, ACP65-74 & Poly Ala)
  - ✓ 10 very hydrophilic (15-18 res.)
  - ✓ 10 very hydrophobic (15-20 res.)

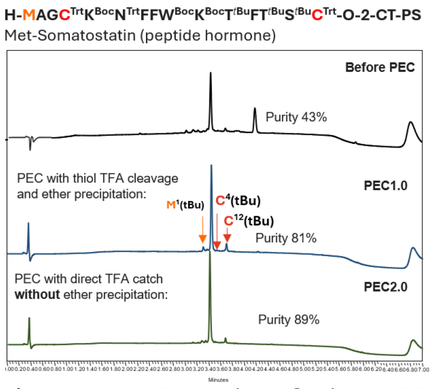


Figure 2. Example of the PEC purification of the peptide hormone Met-Somatostatin by PEC-cartridge.

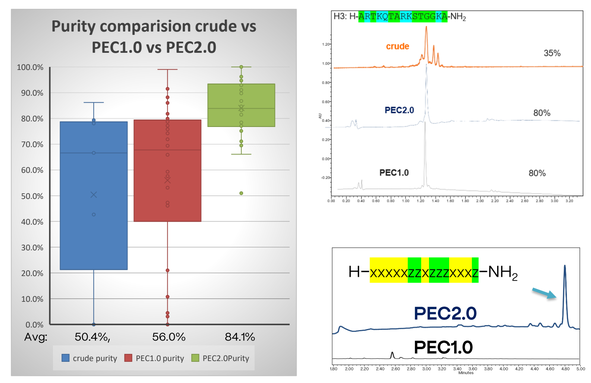


Figure 3. Example of the PEC purification in plate format.

Summary

PEC 2.0 delivers orthogonal peptide purification:

- Handles peptides that chromatography struggles with, including hydrophilic, hydrophobic and aggregating sequences;
- Eliminates ether precipitation and peptide redissolution through direct capture from the TFA cleavage cocktail;
- Supports **flexible scales** and **high-throughput demands**, with up to **96 purifications/day**
- Reduces solvent use to **12 mL per peptide** with the HT kit and saves processing time
- Delivers **84.1% mean purity** across a 32-peptide panel
- Simplifies purification with a streamlined workflow and no gradient development

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SCAN ME

References

[1] R. Zitterbart, et. al., Chemical Science, 2021
[2] R. Zitterbart, S. Lüdtke, M. Muthyala, manuscript in prepartion

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