LIFE OF A PEPTIDE: PREAMBLE TO THE SERIES

A curated journey through the discovery, development, and delivery of peptide therapeutics, guided by voices shaping the future of biotech innovation.

By Charles Johannes

Peptides are transforming from the quiet workhorses of biology into the architects of a new therapeutic era. Once limited by questions of stability and delivery, these elegant molecules now stand at the crossroads of chemistry, biology, and digital science. They are redefining what medicines can achieve and inspiring a generation of innovators to explore the space between small molecules and biologics.

The rise of peptide therapeutics reflects more than scientific progress. It signals a change in how we think about discovery itself. Advances in automation, artificial intelligence, and high-throughput experimentation are shortening the distance between an idea, a drug candidate, and ultimately a final drug product. At the same time, the complexity of peptide development reminds us that success still depends on craftsmanship, collaboration, and a deep understanding of molecular behavior.

Life of a Peptide captures this unfolding story. Through concise perspectives and first-hand insights, the series follows peptides from concept to clinic, revealing the decisions, technologies, and people that shape each stage. Every article explores a pivotal moment in the development process, linking technical discovery to the broader vision of how peptide medicines reach patients.

Whether you're designing a new library, scaling a GMP campaign, or evaluating a platform for investment, we invite you to help illuminate the path from molecular design to human benefit. Join us as a contributor and share your perspective, case study, or insight into any stage of the peptide journey. Beyond the feature articles in the PDHC Chronicle, we've outlined the broader "Life of a Peptide" journey, and details of each stage will be available on the PDHC website.

Overcoming the Peptide Purification Bottleneck: The Evolution of Catch-and-Release Purification

By Dominik Sarma, Elizabeth Denton, Gordon Carlson, and Charles Johannes

1.0 The Persistent Challenge: Synthesis-Purification Mismatch

The field of peptide discovery and development faces a critical operational challenge; a profound mismatch between the high-throughput capacity of modern synthesis and the slower, more labor-intensive nature of traditional purification. Automated parallel synthesizers can produce hundreds of unique peptides in a single run. Yet, purification by Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) typically processes samples one at a time. This disparity creates a bottleneck that slows the discovery-to-clinic timeline for peptide therapeutics.

In this life of a peptide update, we explore peptide purification using the catch-and-release strategy, featuring insights from Dominik Sarma, co-founder of Belyntic and now Product and Market Manager at Gyros Protein Technologies. Belyntic pioneered reductively cleavable linker systems, now commercially known as PurePep EasyClean (PEC™) for the broader peptide community.[1,2] We also highlight recent advances in this field, including Gyros Protein Technologies' newly launched PEC 2.0 technology, which represents the next evolution in catch-and-release, parallel peptide purification.

1.1 The HPLC Reality: Universal Access, Serial Constraints

RP-HPLC remains the gold standard for peptide purification, offering exceptional resolution and the ability to achieve ultra-high purity. Its universal presence in laboratories reflects decades of optimization and familiarity. However, HPLC is fundamentally a serial process. While state-of-the-art equipment can purify a single peptide in 15 minutes, conventional setups are typically limited to 3-4 runs per day, including preparation, method development for difficult peptides, fraction collection, and pooling. When laboratories need to purify 10 or more peptides for a validation study, this translates to several days of sequential processing. Additionally, certain peptide sequences present specific challenges for chromatography. Very hydrophobic peptides often won't dissolve in the water-acetonitrile mixtures required for HPLC injection—a

phenomenon familiar to any peptide chemist who has stared at an insoluble pellet. Very hydrophilic peptides present the opposite problem, flushing straight through RP columns with minimal retention. These edge cases often require extensive method development or entirely alternative approaches. When considering a panel of hundreds of molecules, synthesized in parallel, the probability exists that multiple molecules fall into these edge extremes, restricting the use of a “universal” plug-and-play HPLC protocol.

1.2 The Parallel Alternative: Catch-and-Release Fundamentals

Catch-and-release (c&r) purification, a concept introduced by Bruce Merrifield and co-workers,[1] offers a fundamentally different approach; one deliberately designed for parallel processing. Rather than separating peptides by their chromatographic properties, c&r systems use chemo-selective capture: the target peptide is specifically grabbed while impurities are washed away. This orthogonal separation mechanism enables simultaneous purification of multiple peptides using a single protocol.

The general workflow follows five key steps (Figure 1):

1. Tag installation: During solid-phase peptide synthesis (SPPS), a purification tag is selectively installed on the N-terminus of the full-length peptide. Using a capping step during synthesis ensures that truncated sequences do not contain this tag.

2. TFA cleavage and redissolution: The tagged peptide, along with capped truncations, is cleaved from the SPPS resin with TFA.

3. Immobilization (the "Catch"): The crude peptide mixture contacts a solid support (typically functionalized beads) that covalently reacts with the purification tag, immobilizing only the target peptide.

4. Washing: The solid support is thoroughly washed to remove non-tagged impurities, including truncated sequences, salts, and debris from protecting groups.

5. Cleavage (the "Release"): A specific chemical or physical stimulus cleaves the linker, liberating only the purified peptide from the solid support.

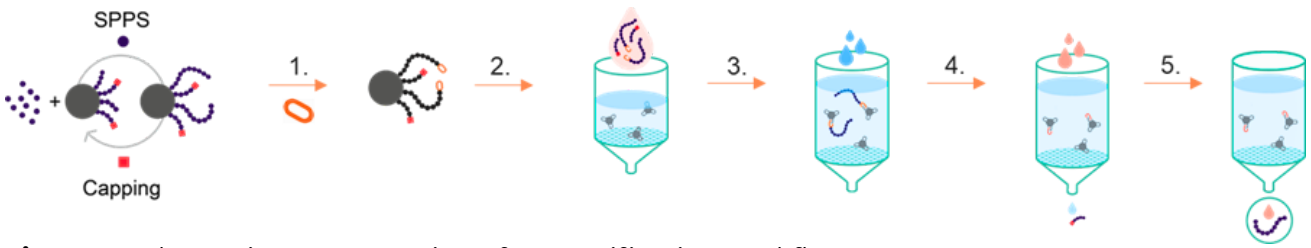

Figure 1. Schematic representation of c&r purification workflows.

This parallel processing capability represents a significant advantage. Where HPLC requires days to complete batch sizes of more than 10 peptides sequentially, c&r approaches can process them simultaneously in one day. Perhaps more importantly, the setup and preparation for a parallel purification approach is significantly less than that required for sequential, even minimally optimized, purification processes. The trade-off, though, is purity: c&r typically delivers what might be termed "fit-for-purpose" purity.

Table 1 gives an overview of purity recommendations for a selection of applications. In the most general sense, c&r can be expected to deliver an average purity of greater than 70%, which is sufficient for many discovery applications where throughput and speed are most critical, and not reliably reaching the 95% high-purity threshold is inconsequential.

Table 1. UV purity recommendations for a representative set of peptide-based applications.

Typical UV-purity (210 nm)	Application
Crude purity	• Initial screening • Sequence optimization • Qualitative binding
Low purity (>70%)	• ELISA testing • Polycolonal antibody production • Peptide arrays
Medium purity (>85%)	• Epitope mapping • Semi-quantitative enzymatic assays • Ligands for affinity purification
High purity (>95%)	• *In vitro* biological assays • Quantitative binding studies • Quantitative inhibition studies
Very high purity (>98%)	• GMP production • Clinical trials • cosmetics

2.0 The Evolution of Linker Chemistry

The effectiveness of any catch-and-release system hinges on its linker chemistry. The ideal linker must satisfy stringent, sometimes contradictory requirements: survive harsh synthesis conditions, enable efficient capture, wash cleanly, and release the peptide under conditions that don't damage sensitive sequences or introduce contaminants. The evolution of linker technologies reflects the challenge of meeting all these criteria simultaneously.

2.1 First Generation: Base-Labile Systems and Their Limitations

Early catch-and-release systems often relied on base-labile linkers, which created a fundamental dilemma. The cleavage conditions—typically pH >11—could damage peptides through several well-documented side reactions such as oxazolidinone and Aspartimide formation (Figure 2)[2]:

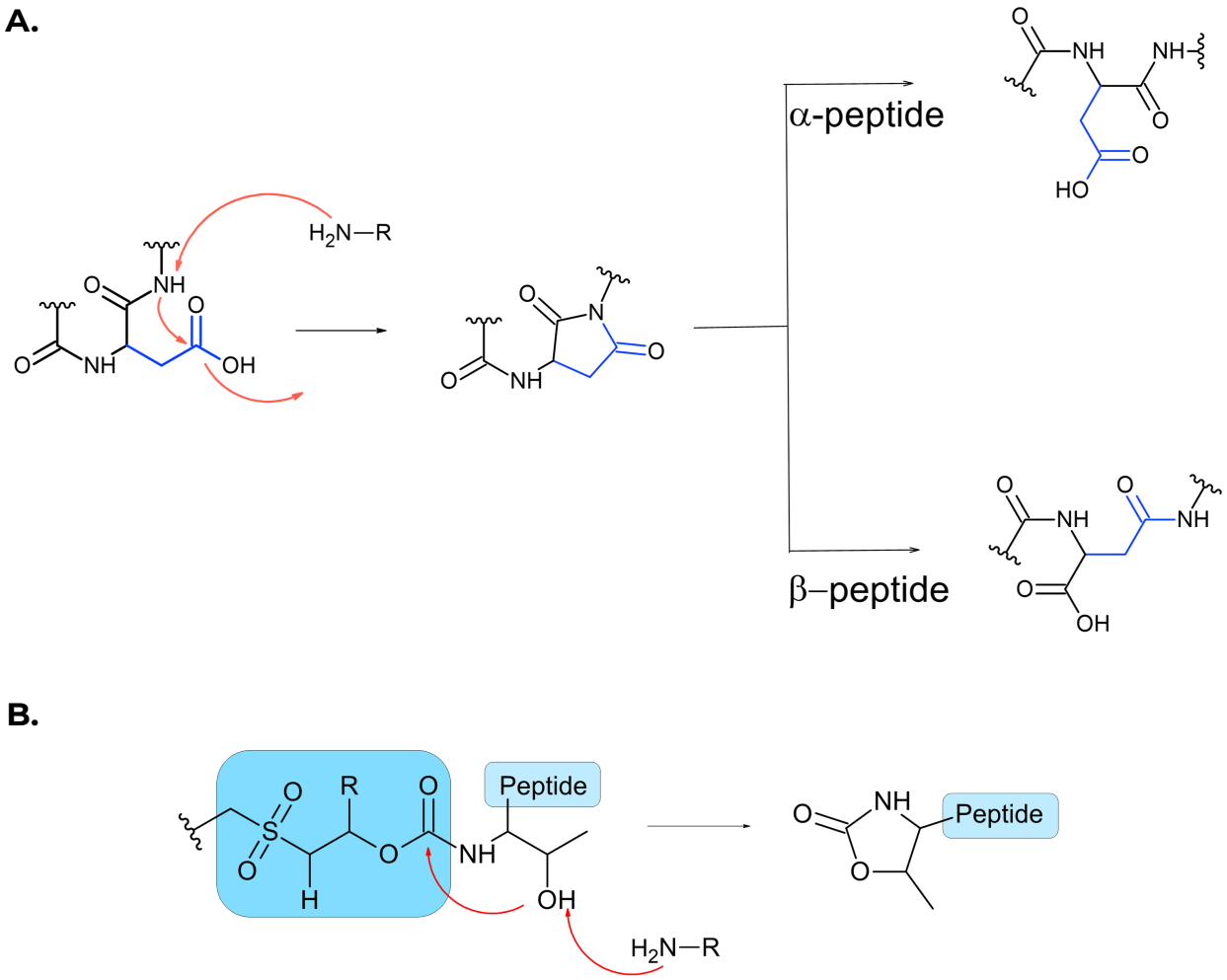

Figure 2. Common side-products using base-induced linker cleavage. A) Aspartimide formation B) Oxazolidinone formation

Chemists faced an un-winnable choice: use harsh conditions that risk damaging the peptide, or use milder conditions that leave the peptide contaminated with cleavage byproducts. Other early systems using nucleophile-induced cleavage introduced different contaminants that often necessitated a subsequent HPLC polishing step—defeating the purpose of parallel purification.

2.2 Reductive Cleavage: The Belyntic Innovation

Dominik Sarma and colleagues at Belyntic pioneered a breakthrough approach using reductive cleavage chemistry, which ultimately led to the invention of the PurePep EasyClean (PEC) technology.[3] Their linker (Figure 3) features a preactivated (grey box) para-azido-benzyl carbamate core (dark blue box) with a protected oxyamine catch tag for immobilization (light blue box). The key innovation lies in the release mechanism: reduction of the aryl azide to an aniline triggers a spontaneous 1,6-elimination cascade that cleaves the carbonate bond.

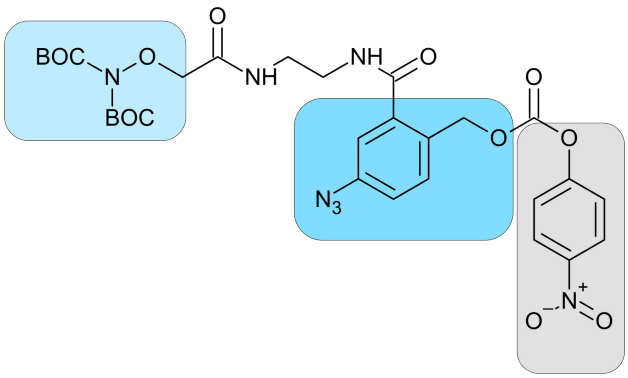

Figure 3. Molecular structure of the first-generation reductively cleavable PEC-Linker.

However, the first-generation linker revealed an unexpected vulnerability: instability in the TFA cleavage cocktail used during peptide deprotection. This instability caused premature linker loss and low peptide recovery, indicating that linker stability optimization was necessary.

2.3 Engineering Stability: The Brominated Linkers

To improve TFA stability while maintaining clean release, the Belyntic team introduced electron-withdrawing bromination.[3] The mono-bromo and di-bromo linkers showed dramatically improved acid stability—92% and 97% intact after 2 hours in TFA cleavage cocktail, respectively.

The mono-bromo PEC linker enabled an elegant "safety-release" mechanism (Figure 4). Using dithiothreitol (DTT), a non-hazardous reducing agent, the azide is reduced at pH 8, "arming" the linker. Crucially, the armed linker remains stably bound, allowing for the complete washout of DTT and its byproducts. An acid wash then triggers clean peptide release. This two-stage approach eliminates contamination from reducing agents while operating under universally compatible conditions. Critically, the carbamate moiety spontaneously decomposes into CO_2 and the free peptide, leaving no residual artifacts—a truly "traceless" release.

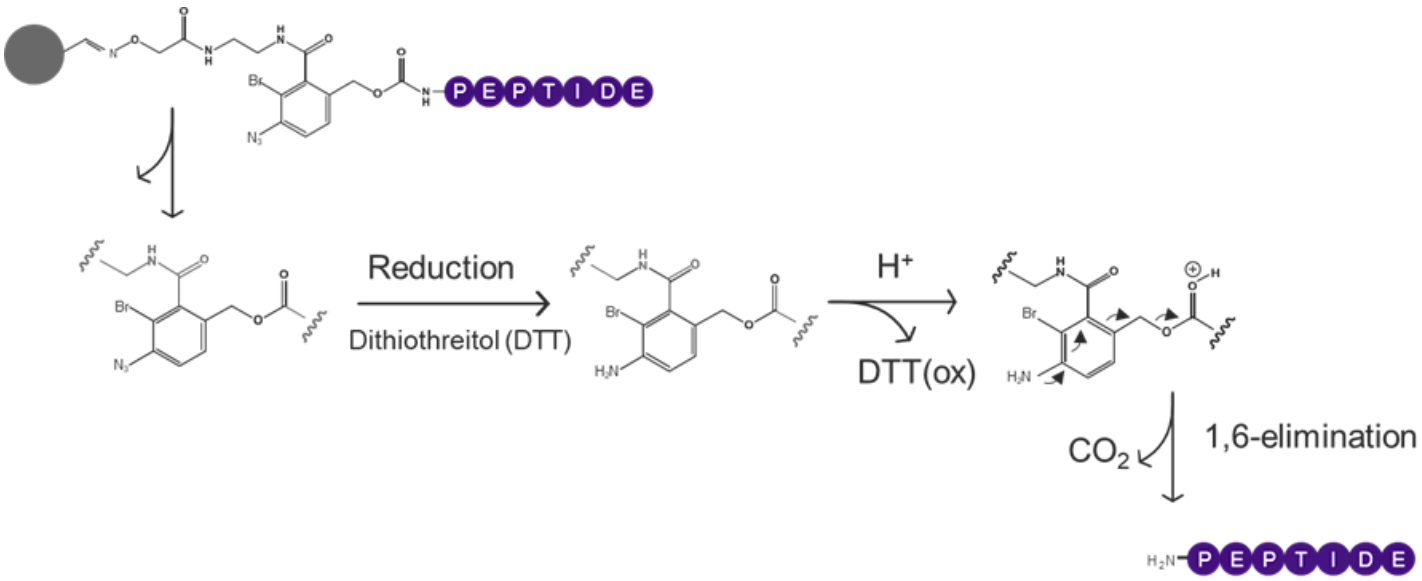

Figure 4. Schematic representation of the safety-release mechanism, enabled through bromination of the aryl-azide core.

The practical impact was demonstrated in a study on neoantigen vaccine manufacturing, where 20 distinct peptides were purified in parallel within 6 hours. One particularly challenging hydrophobic peptide was improved from 6% crude purity to 99% final purity —a result that would have been exceptionally difficult to achieve via traditional HPLC. Even with these impressive time and purity improvement results, it is critical to note that a modest level of purity improvement using c&r purification is required for neoantigen vaccine manufacturing. The presence of related, peptidic impurities, such as n-1 and n+1, in particular, renders many of these assays and products unusable and is among the most challenging impurities to remove via HPLC – a simple task for c&r purification, though.

2.4 PEC 2.0: Eliminating Workflow Steps (Engineering stability – choice of immobilization media)

On November 4, 2025, Gyros Protein Technologies launched PEC 2.0, a significant advance in catch-and-release purification.[4]

Where Belyntic's innovation focused on linker-chemistry optimization, Gyros Protein Technologies' further development of PEC addresses a critical workflow challenge. Former catch-and-release methods, as well as traditional HPLC, require an intermediate step: after TFA cleavage and side-chain deprotection, peptides are precipitated in ether, dried, and then redissolved before immobilization or injection. This redissolution step frequently failed with hydrophobic or aggregating sequences—creating a "success-or-failure" moment that limited downstream reliability.

PEC 2.0's innovation centers on TFA-stable beads (polymethacrylate, as opposed to the agarose used in the previous version) that enable direct immobilization from the TFA cleavage cocktail itself. The workflow simplifies significantly (Figure 5):

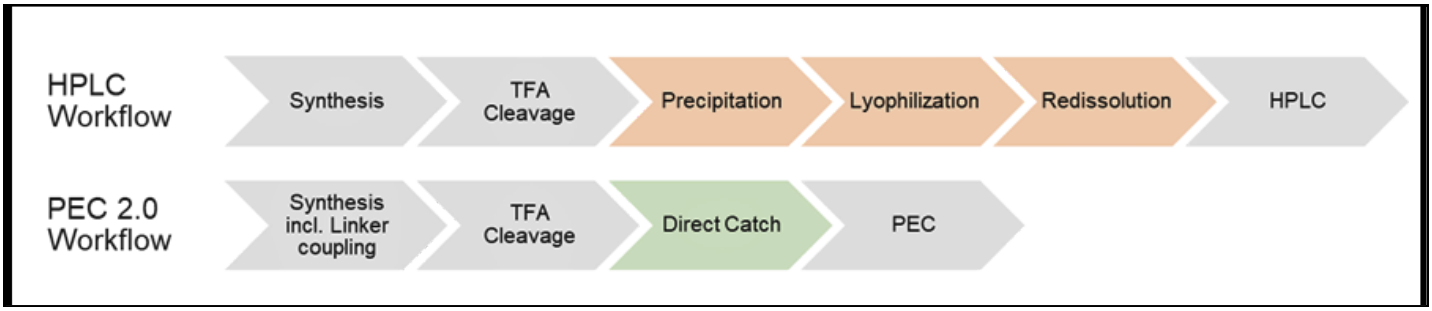

Figure 5. Comparison of conventional HPLC workflows in contrast to the precipitation- and redissolution-free workflow from synthesis to purification with PEC 2.0.

This "redissolution-free workflow from synthesis to purification" eliminates the step where many difficult peptides previously failed. Hydrophobic sequences that won't dissolve, aggregating peptides, and long modified sequences all bypass the redissolution challenge entirely. It's worth noting that a final precipitation is still required after PEC purification for lyophilization—the innovation specifically targets the pre-purification redissolution struggle.

In a recent presentation at the Boulder Peptide Symposium,[5] Dominik Sarma shared results from a case study with T-Therapeutics, a UK-based biotechnology company, showing how PEC 2.0 was used to successfully purify extremely hydrophobic peptides with high Eisenberg values >5 to deliver a set of epitopes for T-cell validation studies – in contrast to the previous vendor that failed to provide all peptides.

3.0 Practical Considerations and Complementary Approaches

The evolution of catch-and-release technologies has not eliminated HPLC—nor should it. Each approach offers distinct advantages that complement different phases of the drug discovery funnel effectively. Many laboratories find value in using both strategically, either as independent purification strategies or in combination.

3.1 When to Choose PEC

Catch-and-release excels in specific contexts:

- Eliminating HPLC bottlenecks via parallel processing: PEC shines when your workflow requires multiple peptides purified at once. Its parallel operation allows you to complete, in a single day, what traditional HPLC would stretch over several days — removing throughput constraints without adding extra HPLC systems.

- Difficult peptides: Very hydrophobic sequences that won't dissolve for HPLC injection, very hydrophilic peptides that flush through RP columns, and long or heavily modified sequences benefit from PEC's orthogonal separation mechanism.

- Discovery-stage applications: Where fit-for-purpose or PEC-grade purity (validated in functional assays) suffices, PEC delivers results faster with less solvent consumption — typically 12x less per run.

3.2 When HPLC Remains Essential

HPLC maintains clear advantages:

- Ultra-high purity requirements: When you need a purity of greater than 95%, particularly for late-stage development or regulatory submissions, HPLC's resolution is typically necessary.

- Established workflows: When current HPLC methods work well and meet timeline requirements, there's limited incentive to change.

- Universal compatibility: HPLC is compatible regardless of the N-terminal modification status. PEC typically requires a free N-terminus for linker coupling (though alternative coupling sites may be developed for specific cases).

3.3 Hybrid Strategies: The Best of Both Worlds

Increasingly, laboratories employ both technologies strategically. Because PEC and HPLC use orthogonal separation mechanisms—chemo-selective capture vs. hydrophobicity-based separation—they complement each other effectively. One study showed that peptides purified by PEC alone achieved a 79% mean purity, but when followed by HPLC polishing, the combination reached a 94% mean purity across a set of very difficult peptides.[6] The PEC step removed synthesis-related impurities efficiently, improved handling (solubility, etc.) of the semi-purified sample, which then simplified HPLC for final purity refinement.

This orthogonal approach appears particularly promising for peptides advancing through development stages. Early discovery work can use PEC alone for speed and throughput. As peptides advance through a discovery pipeline, the number of molecules decreases, while the quantity per molecule increases, along with final purity requirements. HPLC polishing can then be added to the workflow. This staged approach optimizes both speed and final quality.

3.4 Solvent Reduction: A Practical Benefit

PEC offers substantial reductions in solvent consumption. Traditional preparative HPLC can use several hundred milliliters of organic solvent per purification, depending on the flow rate, run time, and gradient, consuming primarily acetonitrile for the mobile phase and column maintenance. PEC's catch-and-release mechanism uses approximately 50 mL of solvent per peptide, representing a significant reduction, even with highly optimized, short HPLC runs.

This difference scales meaningfully in routine use. Laboratories purifying multiple peptides weekly find that reduced solvent consumption eases both waste disposal logistics and associated costs. For organizations with sustainability commitments or facing regulatory pressure around solvent use, this represents a measurable environmental benefit alongside operational advantages.

4.0 Looking Forward: Continued Evolution

The peptide purification landscape continues to evolve rapidly. Several trends suggest where innovation may focus next:

- Expanded chemistry: Developing linker systems compatible with N-terminally modified peptides would broaden the applicability of PEC. Alternative coupling sites and custom linker development may address this limitation.

- Scale and automation: As peptide therapeutics advance through development, scaling PEC methods to millimolar quantities and integrating them with automated workflows becomes increasingly essential. The fundamental principles that enable parallel processing at discovery scale should translate to manufacturing scales.

- Application-specific optimization: Different therapeutic modalities—from screening libraries to personalized neoantigen vaccines to GLP-1 analogs—may benefit from tailored purification approaches. The field will likely see continued specialization.

- Quality standards: As PEC methods become more widespread, establishing clear quality frameworks for "PEC-grade" purity across different applications will enable researchers to make informed decisions about when each method is most suitable.

5.0 Conclusion: Complementary Tools for Modern Peptide Discovery

The evolution of catch-and-release purification—from the early reductively cleavable linkers to Gyros Protein Technologies' workflow simplifications in PEC 2.0—demonstrates how focused innovation addresses specific technical challenges. These advances haven't replaced HPLC; instead, they've expanded the toolkit available to peptide chemists, offering alternative approaches for situations where traditional methods struggle.

The synthesis-purification bottleneck that Sarma and his team identified remains relevant; however, researchers now have more options for addressing it strategically. For parallel processing needs, for challenging peptides that defy traditional chromatography, and for discovery applications where fit-for-purpose purity is sufficient, catch-and-release offers a validated pathway. When ultra-high purity is essential or when current HPLC workflows serve well, chromatography remains the standard. And increasingly, hybrid approaches leverage the complementary strengths of both technologies. (Figure 6)

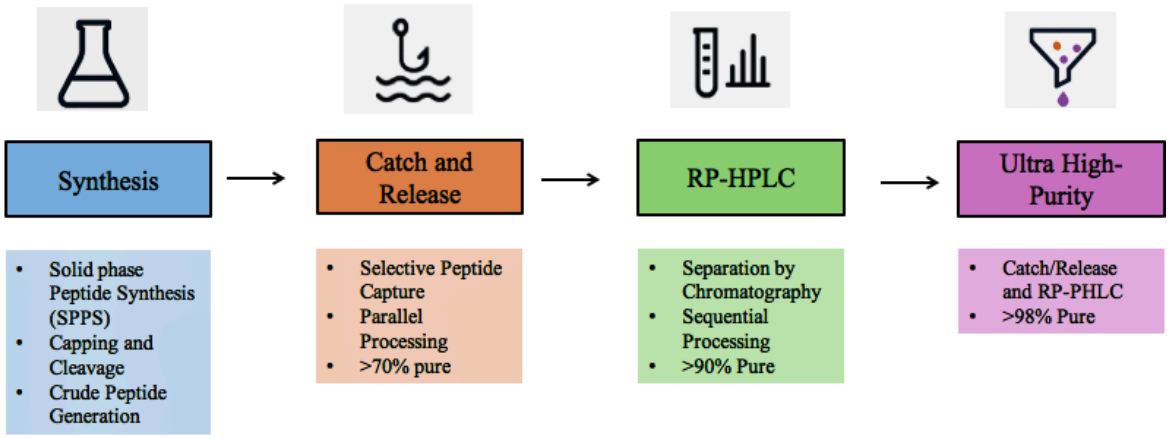

Figure 6. Complementary purification pathways and their roles across the peptide discovery workflow. Peptide synthesis can be followed by parallel catch-and-release purification for speed and throughput (>70% purity), by RP-HPLC when ultra-high purity is required (>90–95%), or by hybrid workflows that combine both approaches to reach >98% purity. This illustrates how PEC and HPLC together address the synthesis-purification bottleneck and support different stages of discovery and development.

As peptide therapeutics continue to achieve clinical success, the field's manufacturing infrastructure must keep pace. The continued innovation in purification technologies, alongside advances in synthesis, analytics, and delivery, ensures that promising peptide candidates can move from concept to clinic with increasing speed and reliability. The next chapter of this story will likely feature further workflow integration, expanded chemical compatibility, and new applications we haven't yet imagined.

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