Targeted purification of GLP-1 therapeutics: Precision X® ligands for Semaglutide and Tirzepatide

Erik Fiedler, Hanna Bobolowski, Heike Böcker, Michael Niemeyer, Florian Settele, Josephine Hammerlindl

Abstract

The ongoing clinical success of incretin-based peptide therapeutics, particularly glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) receptor agonists, has firmly established their therapeutic relevance in the treatment of type 2 diabetes and obesity. These peptides have demonstrated superior efficacy in improving glycemic control and promoting weight loss and remain the only class of drugs approved for both indications. Their dual effectiveness, coupled with favorable safety profiles, has intensified pharmaceutical and biotechnological interest in their large-scale production and commercialization. As market demand continues to rise, there is an increasing need for manufacturing processes that are not only efficient but also robust and compatible with current Good Manufacturing Practice (GMP) standards. This need is especially critical when peptide drug precursors are expressed in complex biological systems such as E. coli or yeast.

In this study, we present our initial developments in ligand discovery aimed at the targeted purification of two clinically validated incretin mimetics: Tirzepatide, a dual GIP/GLP-1 receptor agonist, and Semaglutide, a potent GLP-1 receptor agonist. Using Navigo Pure's proprietary Protein A-derived ligand libraries, we identified a set of affinity ligands tailored for the selective capture of these therapeutic peptides. These ligands were evaluated in the context of clarified E. coli lysates, and were characterized in terms of their binding affinity, target specificity, and overall capture performance.

Our findings demonstrate that the selected ligands effectively enrich the target peptides while minimizing non-specific interactions, establishing a foundation for streamlined purification workflows. The underlying ligand platform developed by Navigo Pure is designed to extend the advantages of Protein A affinity chromatography, traditionally applied to antibody purification, to the broader space of non-antibody biologics. Through our Precision Capturing® technology, we offer a novel and proprietary approach that enables simplified process architectures while maintaining high standards for yield and purity. This platform is particularly well-suited for the purification of recombinant peptide therapeutics, where conventional methods often fall short in scalability, reproducibility, or regulatory compliance.

By enabling a more predictable and scalable purification strategy, Precision Capturing® provides a powerful tool for bioprocess development teams aiming to meet the rigorous demands of clinical and commercial manufacturing. It offers not only a high degree of operational robustness, but also flexibility in adapting to diverse expression systems and process scales. This positions our platform as a unique solution for next-generation biologics manufacturing, supporting the advancement of novel peptide-based therapeutics from research through to GMP-compliant production.

Precision Capturing® Technology

Navigo Pure specializes in the development of custom affinity ligands and proteins through its proprietary Precision Capturing® technology. This advanced platform excels in engineering high-affinity binding molecules for both antibody and non-antibody targets, offering unparalleled flexibility and specificity.

Precision X®

Benefits of Precision X® are no longer limited to just antibody purification:

- Structure based in-silico design
- Prism-like structure with different binding interfaces
- Residues on these interfaces are selected for randomization
- Library complexity > $1*10^{12}$
- Currently more than 20 libraries

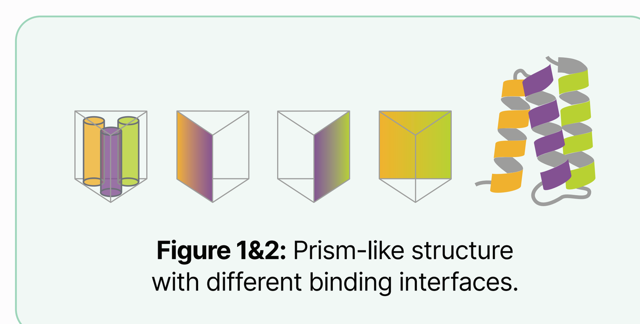

Figure 1&2: Prism-like structure with different binding interfaces.

Ligand Generation & Resin Delivery

The Precision X® ligands were identified by screening libraries with diversities $>10^{12}$ using Phage Display. Ligands with ideal characteristics were coupled to agarose beads for determination of affinity interaction chromatography (AIC) specifications like capacity, elution condition, caustic stability and logarithmic reduction value (LRV).

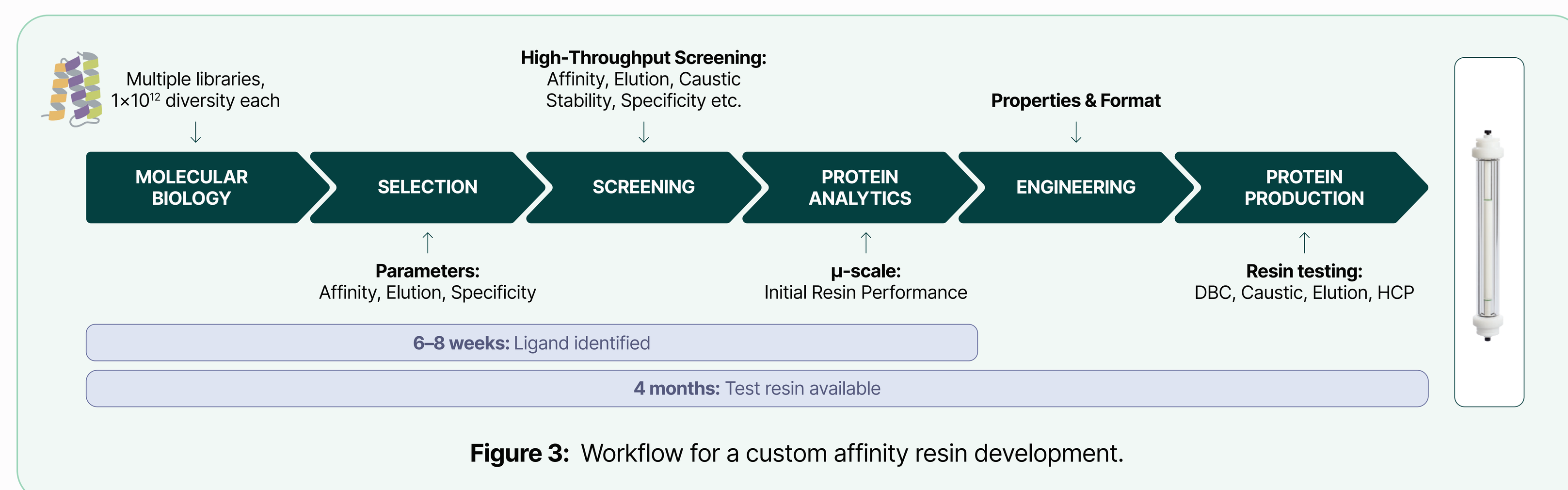

Figure 3: Workflow for a custom affinity resin development.

Results

Successful selection from diverse set of libraries

We employed Semaglutide or Tirzepatide targets immobilized on magnetic beads on a ThermoFisher Kingfisher device to rapidly select pools of enriched binders out of our unique set of ligand libraries. After 3 rounds of Phage display with up to 24 parallel selections , we were able to identify promising libraries with excellent binding to Tirzepatide or Semaglutide.

Precision X ligands bind to Semaglutide or Tirzepatide

After successful selection of ligand candidate pools, we move to our high-throughput automated screening platform, which has the capacity to process ~ 14,000 clones/day, ensuring diversity of hits for our clients. Using the PhyTip from Biotage to generate µg amounts of highly pure ligand, we were able to confirm highly specific and tight binding to Semaglutide or Tirzepatide by Surface Plasmon Resonance SPR (Fig 4).

VARIANT	KD vs TIRZEPATIDE	KD vs SEMAGLUTIDE
Ligand 1	No binding	65 nM
Ligand 2	No binding	34 nM
Ligand 3	No binding	98 nM
Ligand 4	320 nM	No binding

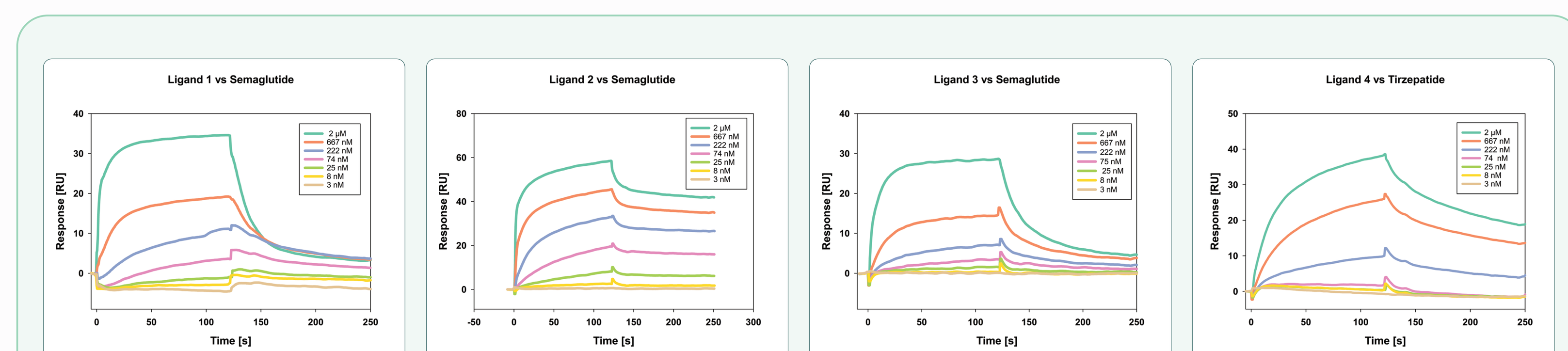

Figure 4: A set of Precision X® ligand candidates exhibit suitable and specific affinities towards Tirzepatide or Semaglutide in surface plasmon resonance (SPR) assay.

Affinity Chromatography Performance of Selected Ligands

The identified Precision X® ligands were purified and coupled on epoxy-activated agarose beads for comprehensive evaluation. Key performance parameters, including dynamic binding capacity at 10% breakthrough (DBC_{10}), elution efficiency, and caustic stability, were assessed in initial experiments without any process optimization performed to date.

VARIANT	DBC_{10} VS SEMAGLUTIDE [mg/ml]	DBC_{10} VS TIRZEPATIDE [mg/ml]	CAUSTIC STABILITY (0.1 M NaOH, 10h) [%]	DBC_{10} [nmol/ml]	CALCULATED DBC_{10} with MW of ANTIBODY	HCP REDUCTION from E.COLI LYSATE [LRV]	HCP REDUCTION from S. CEREVISIAE LYSATE [LRV]
Ligand 1	0.7 ± 0.4	n.d.	n.d.	170	25	>4.5	2.9
Ligand 2	0.9	n.d.	84	146	22	>4.5	2.9
Ligand 3	0.7	n.d.	99	170	25	n.d.	3.4
Ligand 4	n.d.	0.7 ± 0.4	n.d.	146	22	>4.5	3.2

Figure 5: Initial specifications of Semaglutide and Tirzepatide specific prototype resins.

Capturing of Semaglutide and Tirzepatide from complex biological feedstreams

As examples, two prototype resins were challenged by capturing of spiked Semaglutide from E. coli lysate or Tirzepatide from yeast feed. Fractions from load, flow through and elution were analyzed by HCP ELISA and SDS-PAGE (Fig 5/6). After this single affinity chromatography step, we could detect excellent purification of the peptides with very high purity (fig. 6) and great reduction of Host cell proteins (HCP) as determined by respective ELISA assays (Fig. 5).

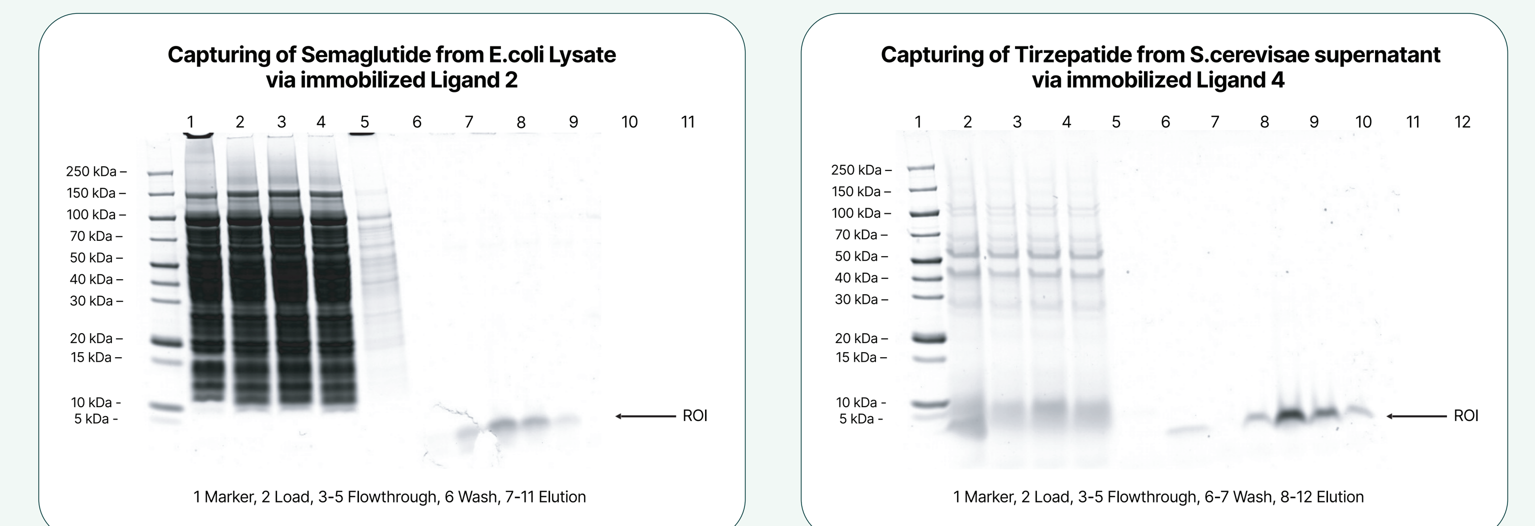

Figure 6: SDS-PAGE analysis of fractions from capturing experiments with spiked E. coli lysates and S. cerevisae supernatant. After sample application and flow through, elution of Semaglutide (left gel) or Tirzepatide (right gel) was performed.

Characterization of eluted Agonists

To evaluate the biological activity of the eluted agonists from spiking experiments, the elution fractions were diluted to PBS buffer and analyzed by its ability to interact with the native GLP-1 receptor using surface plasmon resonance (SPR) analysis (Fig. 7).

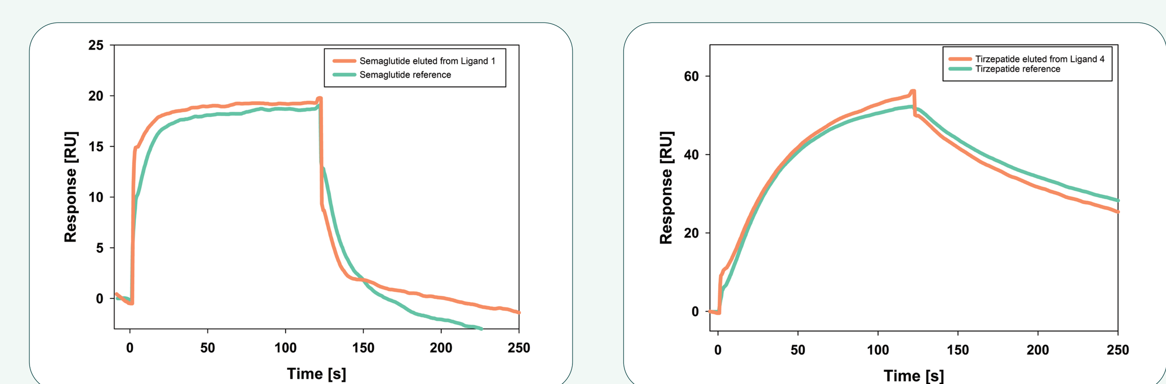

Figure 7: Comparison of binding capability vs GLP-1 receptor of captured and eluted Semaglutide or Tirzepatide from an exemplary prototype resin and commercial references via SPR. Sensograms of eluted samples and reference agonists showed identical binding to GLP-1 receptor, which demonstrated fully active agonists from AIC experiments.

Conclusion

Navigo Pure successfully addressed the challenge of developing affinity ligands targeting the clinically relevant peptide drugs Semaglutide and Tirzepatide. Using our Precision Capturing® platform a rapid, cost-efficient, and sustainable affinity ligand technology, we identified a set of lead candidates with strong and specific binding to both peptides within just 8 weeks. Following an additional 8 weeks, we produced prototype affinity resins and determined initial AIC performance characteristics, demonstrating the feasibility of fast and structured ligand-to-resin development. The ligands showed excellent purification performance, enabling efficient capture of Semaglutide and Tirzepatide from complex biological matrices such as E. coli lysates and yeast-based supernatants.

Furthermore, the eluted products retained their biological activity, as confirmed through receptor-binding assays, affirming their structural and functional integrity. Beyond these specific targets, this work highlights the broader capability of Navigo's Precision Capturing® platform to deliver tailored affinity solutions for non-antibody biologics. By extending the principles of Protein A chromatography into the domain of peptide hormones and other challenging modalities, Precision Capturing® enables robust, scalable, and GMP-compliant purification strategies across a new class of therapeutic molecules. With prototype resins now available, we invite partners to explore collaborative testing and co-development opportunities to advance next-generation bioprocessing.

www.navigo-pure.com

Navigo Proteins GmbH
Business Unit: Navigo Pure, Heinrich-Damerow-Str. 1, 06120 Halle/Germany

josephine.hammerlindl@navigo-pure.com