Affinity based purification of Hyaluronidase by using Precision X® ligands

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Abstract

The enzyme Hyaluronidase catalyses the degradation of hyaluronic acid (hyaluronan), a major component of the extracellular matrix.
In medicine hyaluronidase is used in combination with other drugs to lower the viscosity of hyaluronic acid, to increase the tissue permeability, to speed drugs dispersion and delivery. In cosmetic medicine it's also used to dissolve hyaluronic acid dermal fillers. 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 proteins are expressed in complex biological systems.

In this study, we present our developments in ligand discovery aimed at the targeted purification of Hyaluronidase. Using Navigo Pure's proprietary Precision X® ligand libraries, we identified a set of affinity ligands tailored for the selective capture of this enzyme. The ligands were evaluated in the context of CHO supernatant 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 molecule while minimizing non-specific interactions, establishing a foundation for streamlined purification workflows. The underlying ligand platform Precision X® 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 targets, 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®

Navigo Pure specializes in the development of affinity ligands 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 Protein A are no longer limited to just antibody purification by using Protein A as scaffold for library design:

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

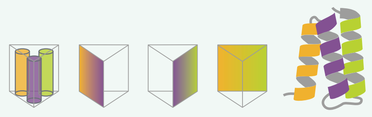


Figure 1&2: Prism-like structure with different binding interfaces

Ligand Generation & Resin Delivery

The Precision X® ligands were identified by screening libraries with diversities > 1*10^{10} 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).

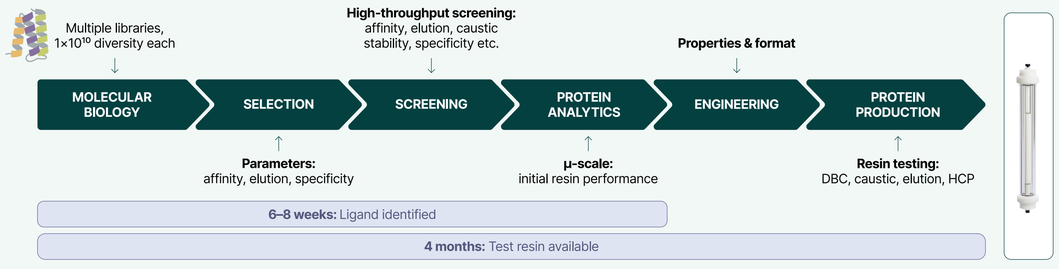


Figure 2: Workflow for a custom affinity resin development

Results

Successful selection from diverse set of libraries

We employed Hyaluronidase 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 12 parallel selections, we were able to identify promising pools from different libraries with excellent binding to Hyaluronidase (Fig 3A).

Precision X® ligands bind to Hyaluronidase

After successful selection of ligand candidate pools, we moved to our high-throughput automated screening platform which has the capacity to process ~ 15,000 clones/day, ensuring diversity of hits for our clients. Using the PhyTips from Biotage to generate µg amounts of highly pure ligand, we were able to confirm highly specific and tight binding to Hyaluronidase by Biolayer Interferometry (Fig 3B). Best candidates were analyzed more deeply after lab-scale purification (Fig 3C).

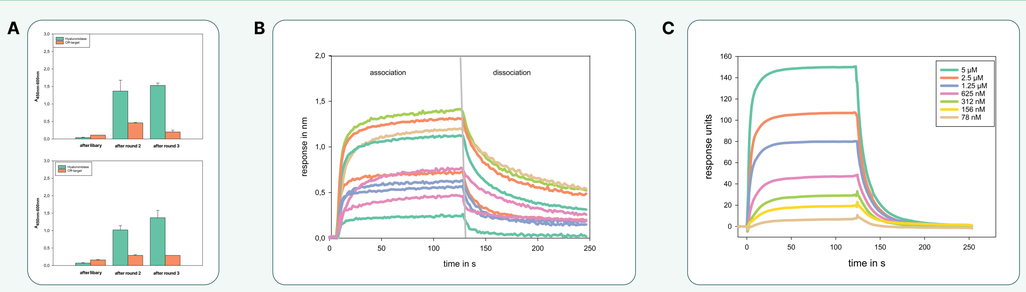
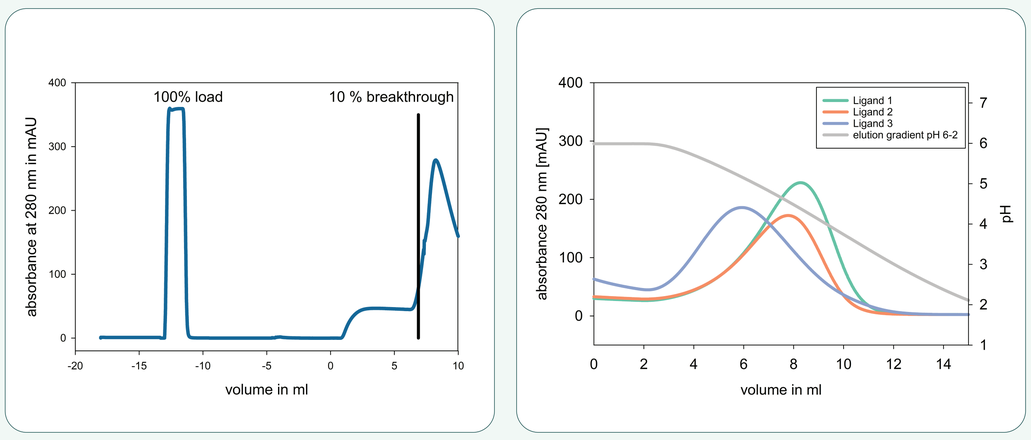


Figure 3: A. Selective pools for Hyaluronidase detected in different libraries. **B.** Specific binding of different ligands from libraries to Hyaluronidase was confirmed in Biolayer Interferometry assay. **C.** Confirmation of binding of purified ligand by SPR assay.

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 pH and caustic stability, were assessed in initial experiments without any process optimization performed to date. After evaluation of the respective ligand candidates, Ligand 3 was selected as lead candidate for prototype resin production, showing good dynamic binding capacity and stability towards sodium hydroxide whilst maintaining enzymatic activity of Hyaluronidase through mild elution at pH5.



	DBC_{10} VS HYALURONIDASE [mg/ml]	CAUSTIC STABILITY (0.1 M NaOH, 10h) [%]	ELUTION pH	ENZYMATIC ACTIVITY OF HYALURONIDASE IN ELUTION
Ligand 1	9	72	4.5-5.0	✓
Ligand 2	7	69	4.5-5.0	✓
Ligand 3	**11**	**100**	**5.0-5.5**	✓

Figure 4: Initial specifications of Hyaluronidase specific prototype resins.
Ligand 3 has been selected as lead candidate for prototype resin production

Capturing of Hyaluronidase from complex biological feedstreams with Ligand 3

After selection of the lead ligand candidate, a prototype resin was produced and challenged by capturing of Hyaluronidase spiked into mock CHO supernatant. Fractions from load, flowthrough and elution were analyzed by SDS-PAGE (Fig 5). Elution fraction shows excellent performance of the prototype resin with a single Hyaluronidase band displaying with high purity. Samples were subjected to HCP ELISA and significant reduction of HCPs was observed with LRV > 3.5.

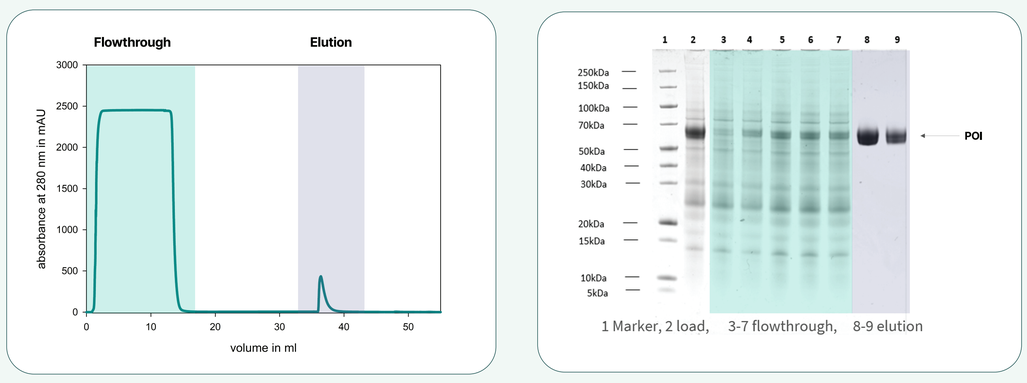


Figure 5: SDS-PAGE analysis of fractions from capturing experiments with Hyaluronidase spike into mock CHO Supernatant. After sample application and wash unbound sample step, efficient elution of Hyaluronidase was performed

Conclusion

Navigo Pure successfully developed affinity ligands targeting the clinically relevant enzyme Hyaluronidase. Using our Precision X® platform a rapid, cost-efficient, and sustainable affinity ligand technology, we identified a set of lead candidates with strong and specific binding to Hyaluronidase 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 Hyaluronidase from complex biological matrices such as CHO supernatant. Furthermore, the eluted products retained their biological activity, as confirmed by Hyaluronidase activity assay, affirming their structural and functional integrity.
Beyond this specific target, this work highlights the broader capability of Navigo's Precision X® platform to deliver tailored affinity solutions for non-antibody biologics. By extending the principles of Protein A chromatography into the domain of enzymes and other challenging modalities, Precision X® 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.

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