

# Overcoming challenges in Ferritin extraction from blood plasma: precise affinity chromatography solutions

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## Abstract

Ferritin is a critical protein complex essential for iron storage and release in living organisms. Ensuring high purity and effective isolation of Ferritin is vital for its various biomedical and industrial applications. However, due to its low presence in human plasma, the purification of Ferritin can be very challenging.

The small amount of Ferritin (13-400 ng/mL) present in plasma makes purification a significant challenge for purification techniques. However, we have successfully overcome this challenge. Our next-gen Affinity Chromatography (AC) methods enable the isolation and purification of Ferritin with exceptional precision, ensuring high purity and yield even from very small initial amounts. This achievement underscores our commitment to leveraging cutting-edge technology to solve complex biochemical problems.

The development of AC for Ferritin purification is a significant bioprocessing advancement. This technique improves the efficiency of Ferritin isolation and ensures the production of high-quality Ferritin for applications in medical diagnostics and therapy. It can serve as a blueprint for many other antibodies, non-antibodies and proteins with clinical potential facing purification obstacles.

The custom affinity resin was developed using the Precision Capturing® technology. The platform brings the benefits of Protein A affinity chromatography to the space of non-antibody proteins. Precision Capturing® enables a simpler process architecture resulting in optimized purity and yield. It is a unique and innovative affinity technology for DSP development to achieve a robust, predictable, scalable, and GMP-compliant purification procedure.

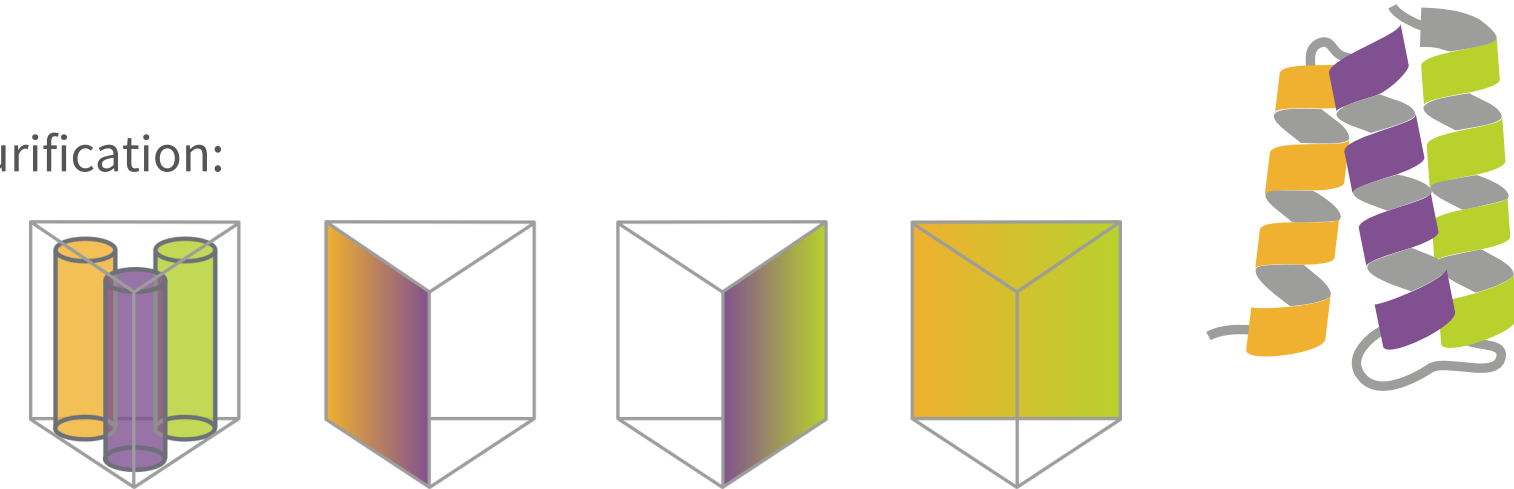
### Precision Capturing® Technology

Navigo Proteins 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.

Benefits of **Precision A®** 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 >10<sup>10</sup>

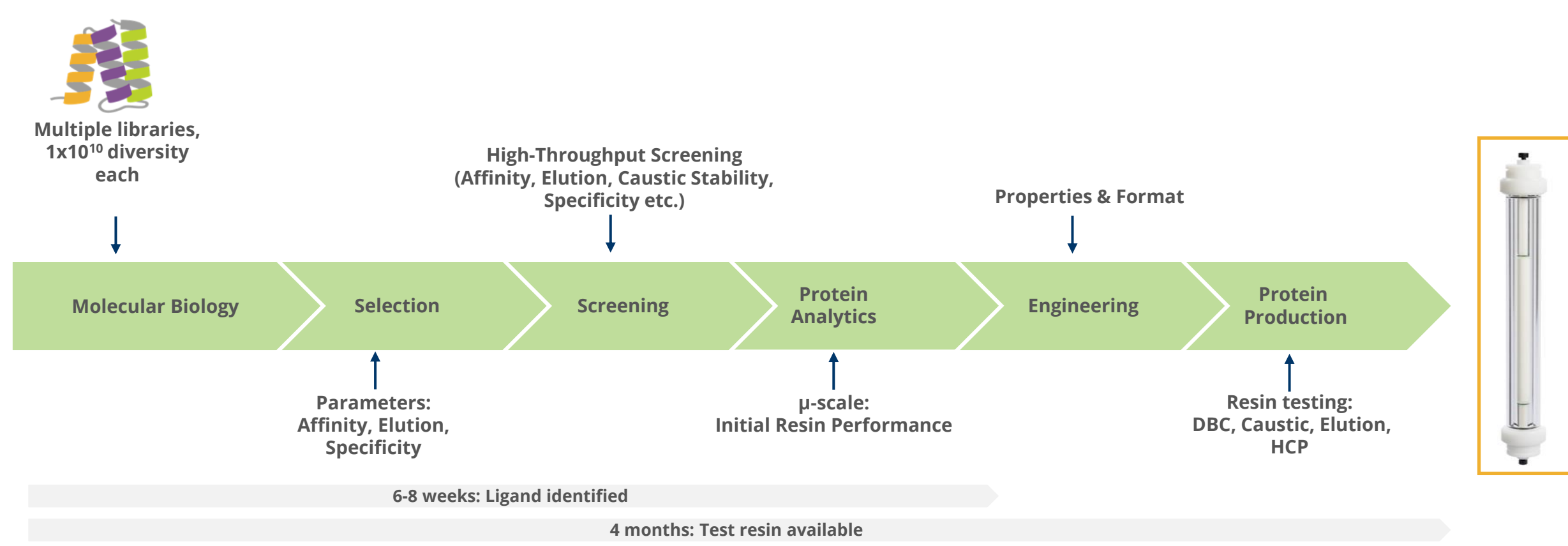
Currently more than 15 libraries



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

### Ligand Generation & Resin Delivery

The **Precision X®** ligand was identified by screening libraries with diversities >10<sup>12</sup>. Ligands with ideal characteristics were coupled to agarose beads for thorough testing. Ligands with the ideal resin are coupled on agarose beads and then scaled-up, validated for large-scale manufacturing in a GMP-validated environment.



**Figure 3:** Workflow for a custom affinity resin development

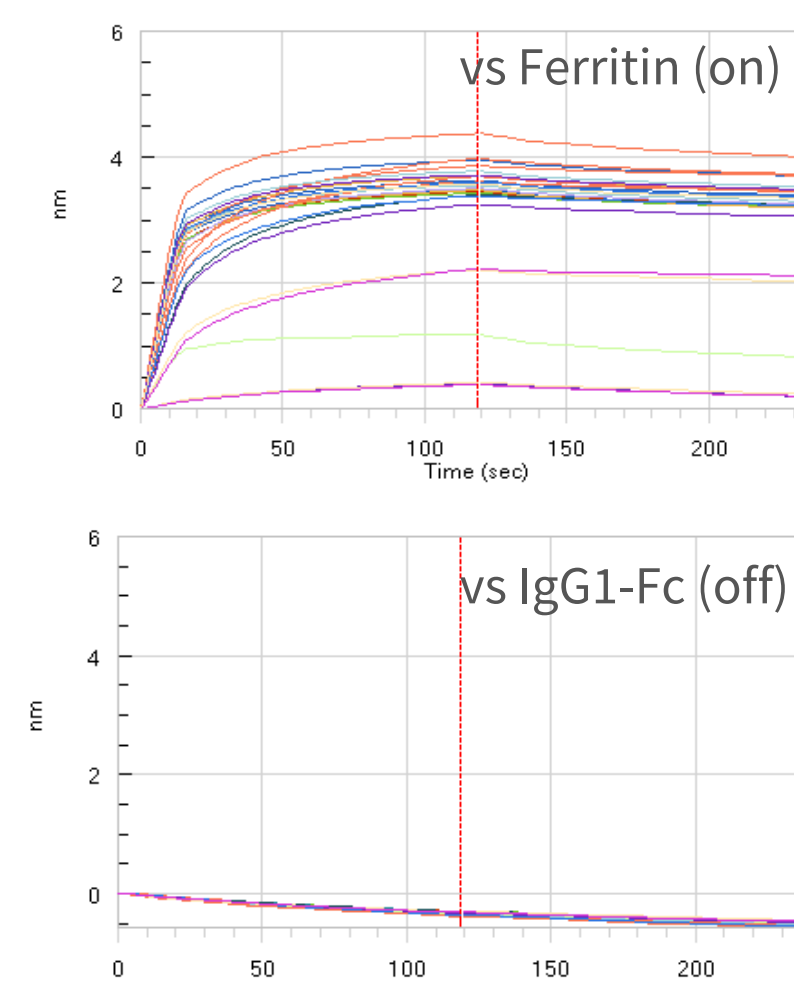
## Results

### Successful selection from diverse set of libraries

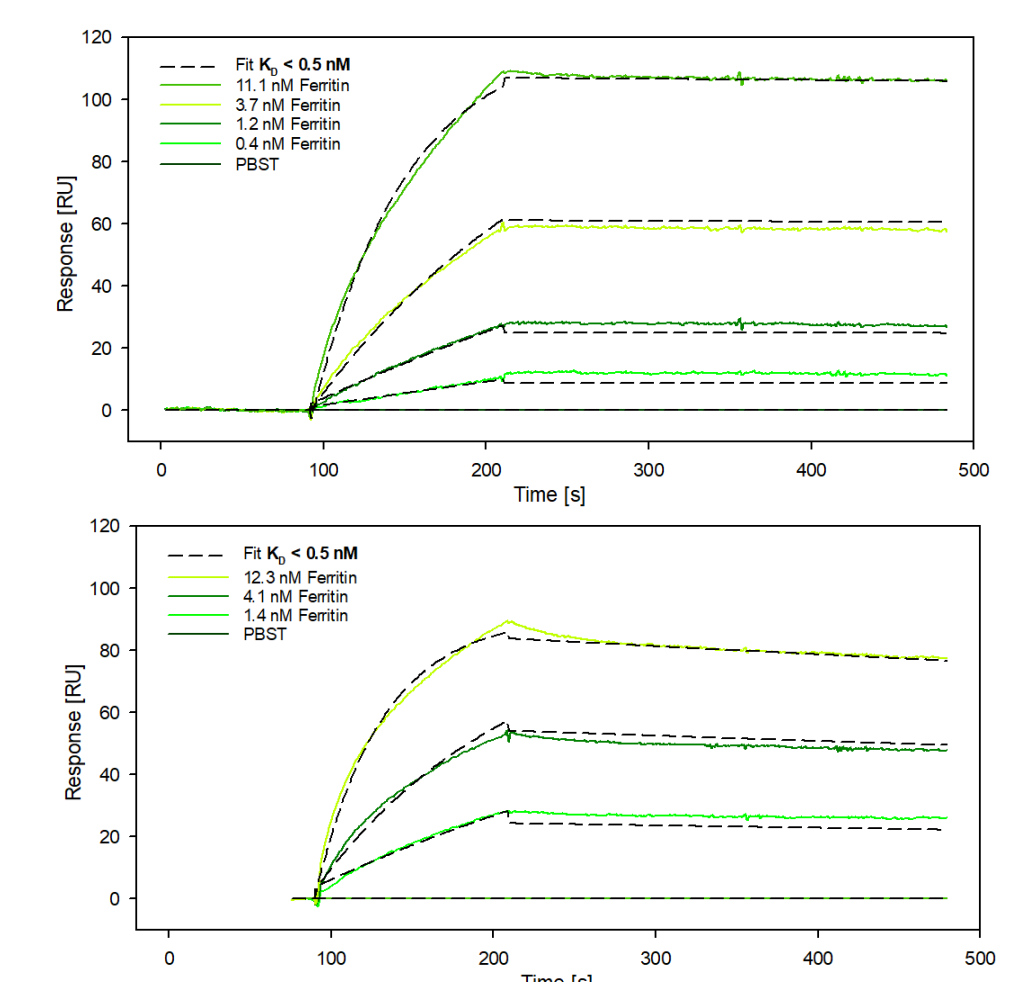
We employed Ferritin target immobilized on magnetic beads on a Thermo Fisher Kingfisher device to rapidly select pools of enriched binders out of our unique set of ligand libraries. After 3 rounds of Phage display with up to 24 parallel selections, we were able to identify promising libraries with excellent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

### Precision X® ligands bind very tightly to Ferritin

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 (KD < 0.5nM) to Ferritin by BLI and SPR (Fig. 4/5)



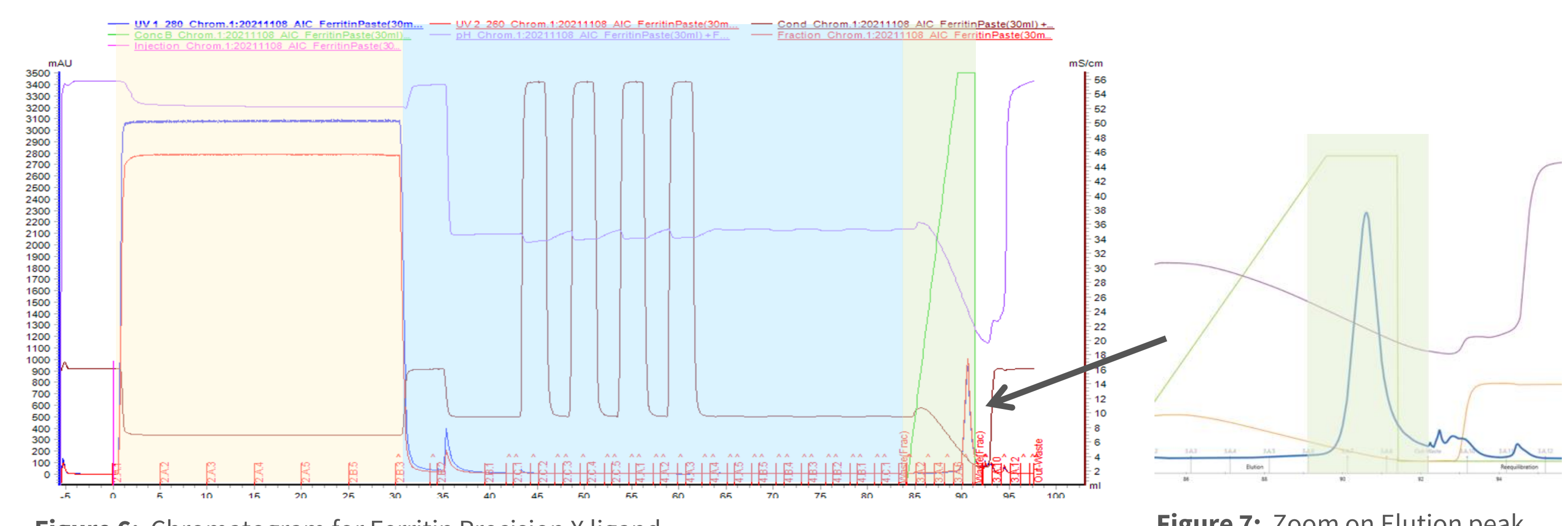
**Figure 4:** Diverse set of variants identified in Biolayer Interferometry (BLI)



**Figure 5:** Precision X® ligand candidates exhibit extraordinary affinity towards Ferritin in surface plasmon resonance (SPR) assay

### Ferritin affinity resin efficiently captures Ferritin from human plasma

The Precision X® lead candidate was coupled to commercial agarose resin and tested chromatographically. Purified Ferritin, spiked into human serum for detection, was applied to the column and after several wash steps, the bound target protein was eluted from the column with a citrate gradient. Overall, Ferritin was efficiently captured and eluted with a sharp peak at approximately pH3, whereas the vast majority of serum proteins (including abundant albumin) remained in the flow-through or wash fractions. This resulted in excellent purity of Ferritin despite low amounts in the serum as evidenced by SDS-PAGE (Fig. 8). Interestingly, the purification process was visually indicated by an orange stain, which appeared during capture of the target protein and disappeared during elution. The pools of the elution fractions displayed the observed orange color in the collector tube. After successful trials at Navigo Proteins, prototype samples were provided to PreviPharma for further verification in blood serum process development.



**Figure 6:** Chromatogram for Ferritin Precision X ligand

**Figure 7:** Zoom on Elution peak

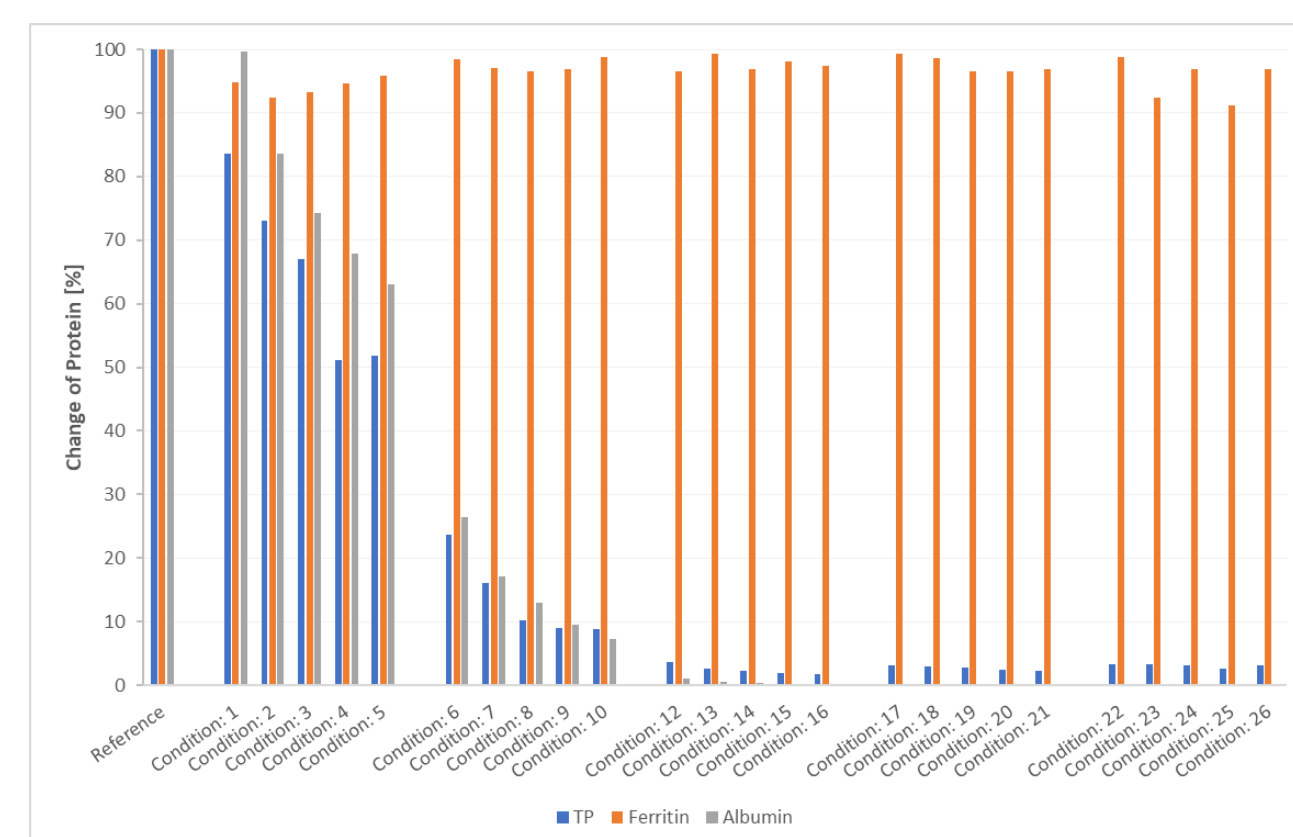
PreviPharma Consulting GmbH is a research company establishing platforms for purification and qualification of human plasma derived medicinal products. Therefore, purification technologies and QC methods have to be developed up to a large production scale.

Here, Ferritin can play an important role as a potential target protein for the therapy of severe anemias, or as apo-ferritin in severe cerebral bleedings.

### Challenges of Ferritin Purification

Extremely low concentrations in plasma and plasma-derived fractions → a special purification strategy had to be developed to provide sufficient Ferritin amounts to perform the positive affinity chromatography [patent has been filed]

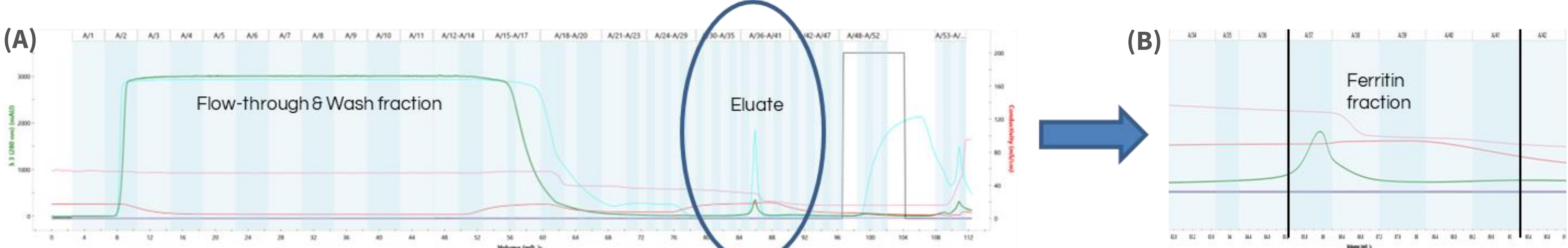
- Large volume quantity of starting material must be reduced beforehand to decrease the run time for the positive affinity chromatography
- Impurity removal step was performed to remove large quantities of unwanted proteins



**Figure 10:** Results for impurity removal step. Reduction of TP and Albumin by a factor of ~32 and ~6000.

### Positive Affinity Chromatography and Results

Ferritin was successfully purified by positive affinity chromatography (CV = 0.5 mL) with high purity and yield.

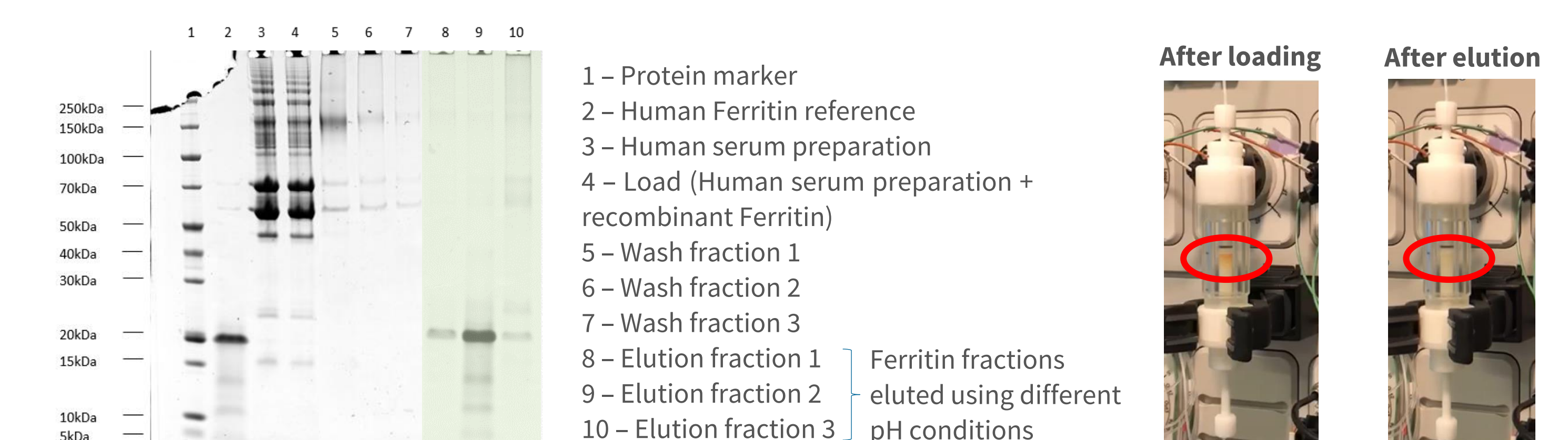


**Figure 11:** Chromatogram for Ferritin purification from plasma & plasma fraction. (A) Original Chromatogram. (B) Zoom of elution peak.

Total protein (TP) and Ferritin content was detected using BCA method and ferritin ELISA (IBL).

Sample	Ferritin [ng/mL]	Ferritin [ng]	Purity [%]	Step Yield [%]
Load	14,418.3	634,405.2	0.04	100.0
Flow-through/Wash	26.7	1,511.2	0.00008	0.2
Eluate	116,126.5	569,019.9	88.6	89.7

Impurities ~11%:  
For example, transferrin and haptoglobin



**Figure 8:** SDS-PAGE of positive affinity chromatography run under reduced conditions (line 8-10)

**Figure 9:** Visual observation of Ferritin bound to the column

## Conclusion PreviPharma

PreviPharma established successfully using the Ferritin affinity ligand a method for the purification of Ferritin from plasma and plasma-derived fractions with high purity and yield. As Ferritin is present in extremely low amounts in the starting material, the resin could not be used without a prior purification strategy. The following challenges could be solved:

- > Development of a purification method for Ferritin with high yield and purity
- > Removal of unwanted proteins by different process steps [patent has been filed]
- > Reduction of large initial volume to minimize the run time for the positive affinity chromatography
- > Such a ligand-based technology can be used for an effective purification, especially for proteins which are present in very low concentrations only

Using the Ferritin affinity resin, Ferritin could be effectively purified in various runs with the following key data:

- > Purity: 89% - 96%, Step Yield: 85% - 95%

## Conclusion Navigo Proteins

Navigo Proteins efficiently addressed the challenge of extracting Ferritin from blood plasma, overcoming limitations of traditional methods. In just 8 weeks, we identified strong Ferritin-binding candidates using our advanced affinity ligand technology - a faster, more cost-effective, and sustainable approach. After 8 more weeks of testing, we supplied PreviPharma with a final resin for Ferritin purification studies. Key outcomes include a high-affinity Ferritin ligand and successful extraction from various liquid sources.

- > Ferritin ligand with excellent and specific binding to target molecule (KD < 0.5nM)
- > Ferritin is efficiently extracted from plasma and serum and can be used for other liquid compositions as well (cell culture supernatants).
- > Prototype material available for testing by interested companies

