

Advantage of antibody based selectivity in the purification of biologics

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Bioprocessing

INTRODUCTION

Advances in bio-therapeutics are generating an increasing range of complex molecules that present unique and often complex purification challenges. By taking advantage of antibody based selectivity, Camelid heavy-chain antibody fragments (V_HHs) have proven to be a reliable immuno-affinity chromatography (IAC) solution in the downstream process of biologics. Thermo Scientific™ CaptureSelect™ affinity products and analytical tools are developed for the discovery and manufacturing of even the most demanding bio-therapeutics. The affinity resins provide high target purity in a single step, independent of feedstock.

CAPTURESELECT TECHNOLOGY – UNIQUE AFFINITY PURIFICATION SOLUTION

- Affinity through antibody selectivity: technology based on Camelid single domain [V_HH] antibody fragments
- Unique V_HH screening technology to determine final resin properties such as target specificity, mild elution & ligand stability
- Animal origin free production process (*Saccharomyces Cerevisiae*)
- Technology used in commercial purification processes

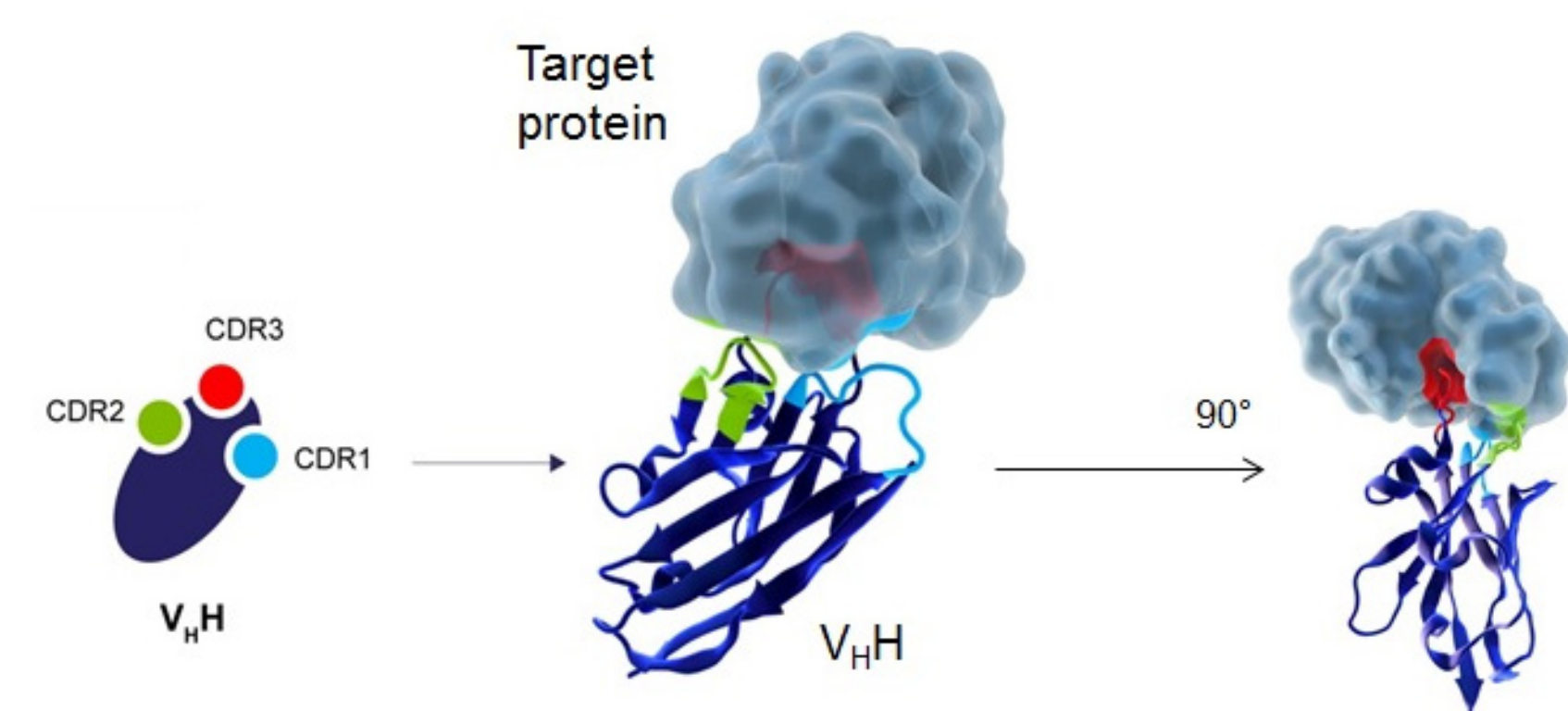
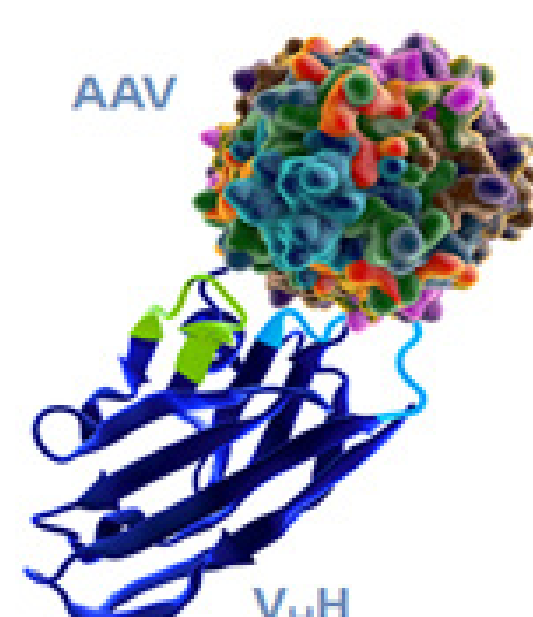


Fig.1 CaptureSelect ligands are V_HH fragments (single domain antibody fragments – sdAb), the smallest antigen binding molecules.

The small size of V_HH fragments (15kD) allows binding at difficult to reach epitopes. Overall, V_HH fragments offer high specificity, affinity and stability.

LARGE SCALE PURIFICATION OF AAV VECTORS

- Increased yields through the reduction of chromatography steps in the viral vector purification process
- High purity and recovery in a single purification step
- Scalable purification of multiple AAV serotypes



| Thermo Scientific™ resin | Binding Capacity (vg/mL) | Serotype Affinity |
|----------------------------|--------------------------|--|
| POROS™ CaptureSelect™ AAV8 | >10 ¹³ | AAV8 |
| POROS™ CaptureSelect™ AAV9 | >10 ¹⁴ | AAV9 |
| POROS™ CaptureSelect™ AAVX | >10 ¹⁴ * | AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, recombinant & chimeric vectors |

* viral genomes per milliliter (vg/mL), binding capacity will vary based on serotype, feed stream, additives, and mutations to parent serotypes

POROS CAPTURESELECT AAVX: A PLATFORM FOR AAV PURIFICATION

- Broad selectivity to both natural and synthetic capsids
- High dynamic binding capacity
- High elution recovery at different flow rates
- Robust, with less process optimization steps

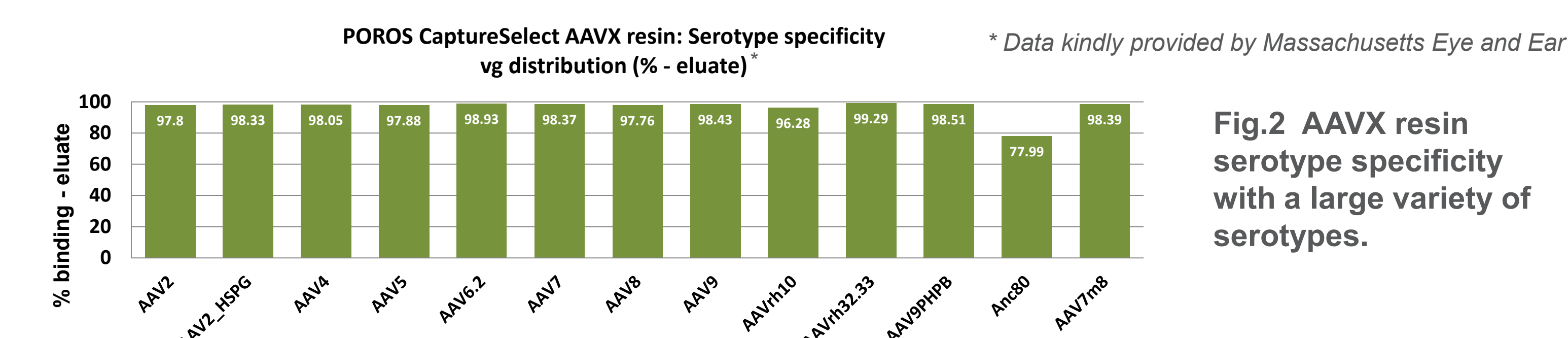


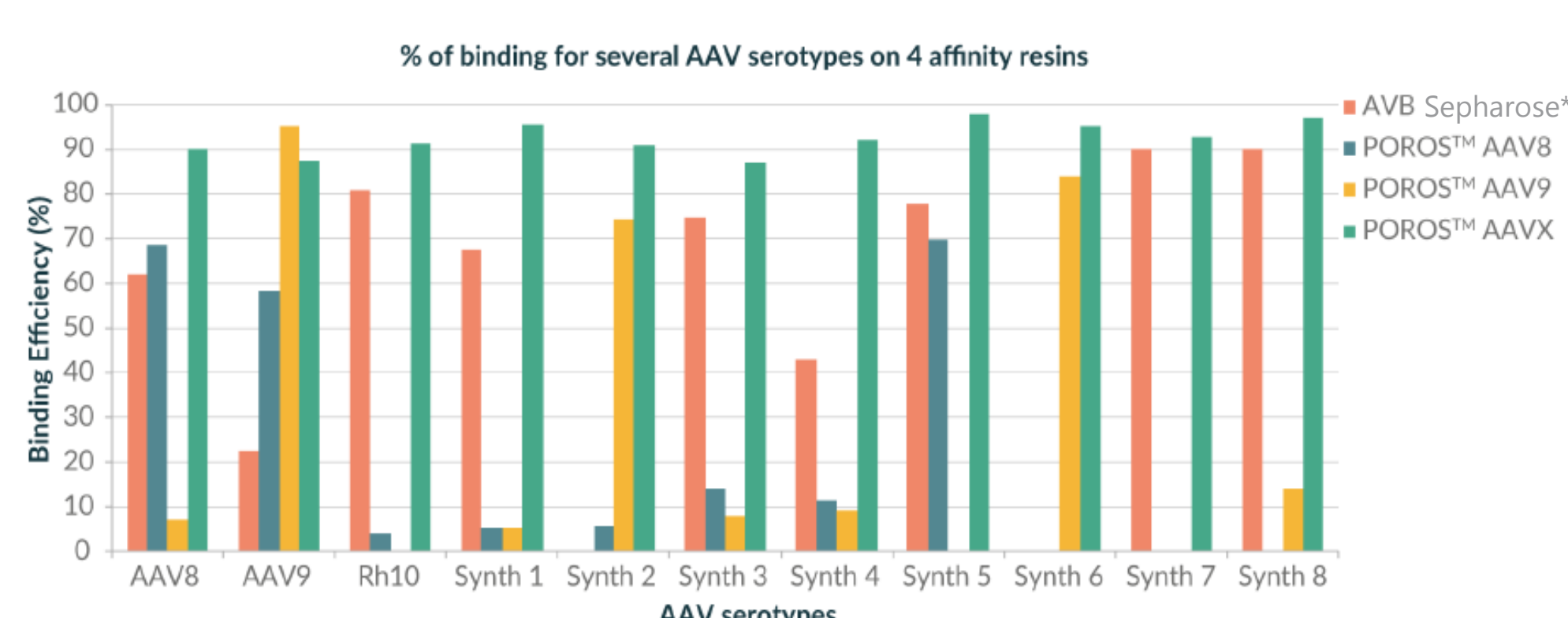
Fig.2 AAVX resin serotype specificity with a large variety of serotypes.

Experimental settings. Static binding mode experiment: resin was mixed with AAV serotype in tube – no flow properties performed. VG was determined by qPCR

The following data has been kindly provided by Genethon and published in *Cell Gene Therapy Insights* 2018; 4(7), 637-645.

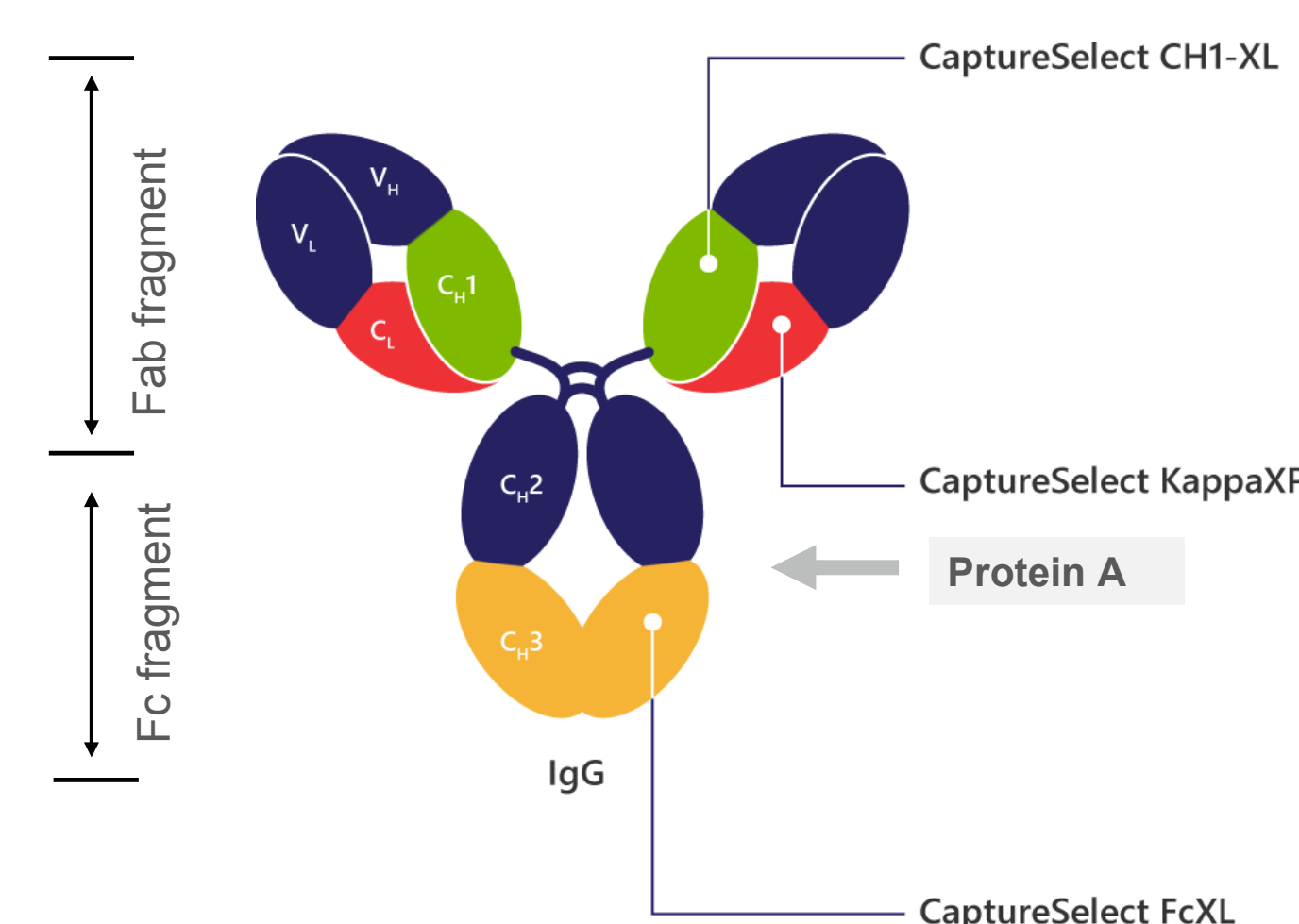
Fig.3 Comparison of POROS CaptureSelect AAV resins with an alternative resin.

3 native AAV vectors (AAV8-10) and 8 synthetic vectors were tested in static binding mode with the 4 different resins.



- ✓ The use of AAV affinity resins simplifies processes and can reduce costs by a factor of 6 (customer testimonial)

PURIFICATION OF ANTIBODY THERAPEUTICS



A unique set of CaptureSelect affinity ligands has been developed (fig. 4.), directed against a variety of antibody subdomains, providing tools for researchers and manufacturers to help facilitate purification of a broad range of antibody formats.

Fig.4 CaptureSelect resins: Antibody Binding Selectivity
Binding regions of CaptureSelect resins for affinity purification of antibodies and antibody fragments.

ANTIBODY PURIFICATION – CAPTURESELECT CH1-XL

- CH1 binding domain ligand
- No co-purification of free light chains (only correct assembled Fabs)
- Efficient elution at milder pH (4 – 4.5)

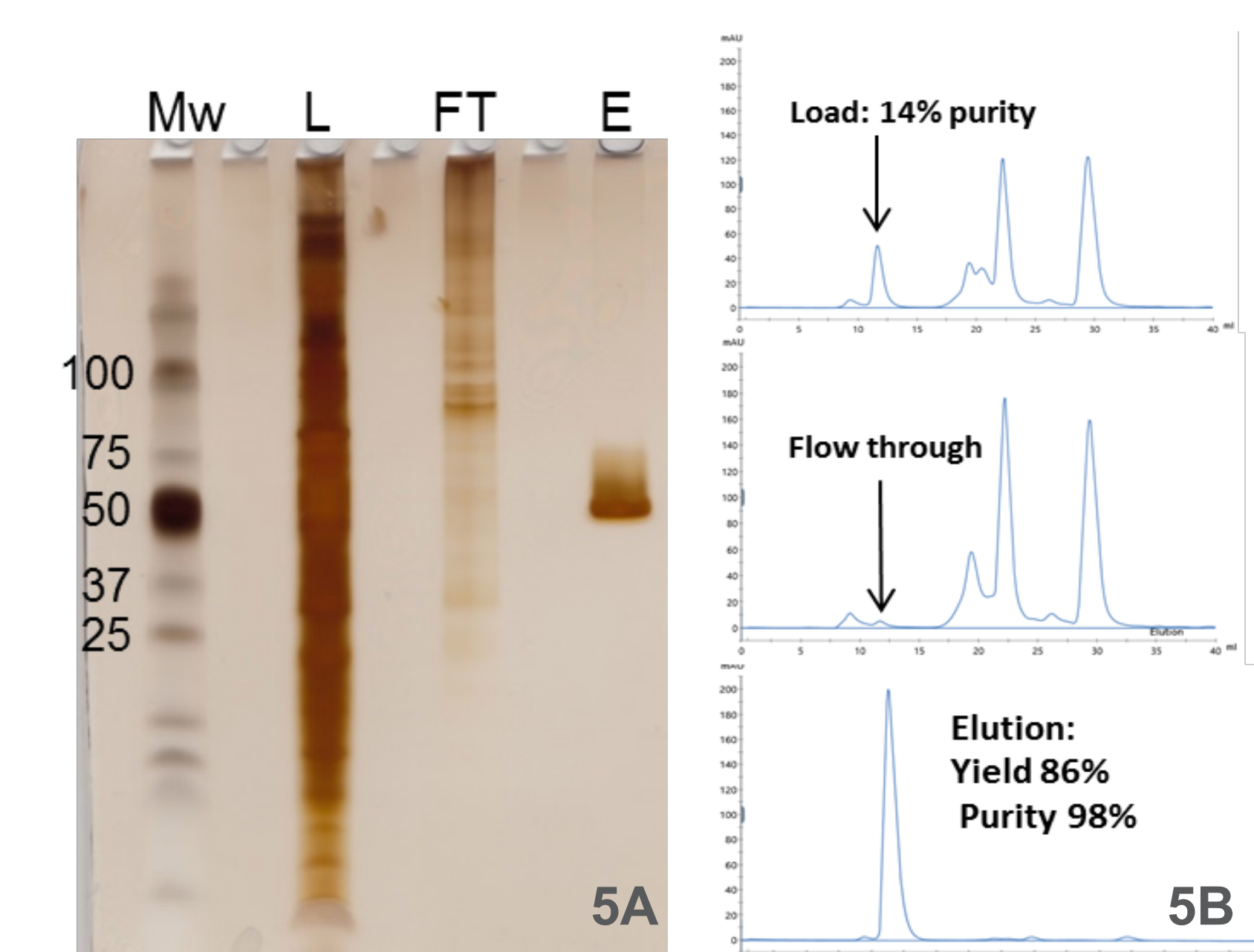


Fig.5 Ranibuzimab feed from HEK293 cells. Analysis of the fractions after purification with CaptureSelect CH1-XL resin shows high yield and purity in a single step.

5A: SDS-PAGE silver staining of the load (L), flow through (FT) and elution (E) fractions, showing no presence of light chains in the elution pool.

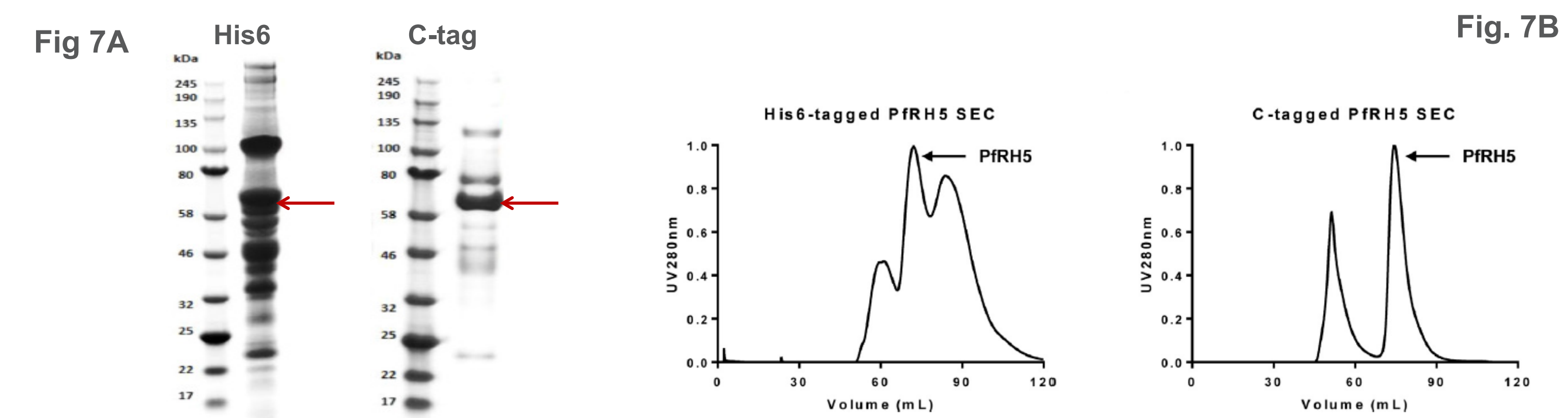
5B: Gel filtration analysis showing 98% purity of the Fab fragment in the elution fraction with a yield of 86%

- ✓ A true platform for Fab fragment purification

C-TAG – A REVOLUTIONARY AFFINITY TAG

- Unique affinity tag based on a 4 residue C-terminal peptide sequence: E-P-E-A
- Limited effect on protein functionality unlike larger tags 9GST, HIS6)
- Enabling high purity and yield from complex mixtures in a “one-step” process
- Mild elution, protecting the protein of interest

cGMP PURIFICATION OF THE PFRH5 PROTEIN-BASED MALARIA VACCINE



| Process yield (after) | His6-tagged construct | C-tagged construct |
|-------------------------------|-----------------------|--------------------|
| Culture supernatant | 100% | 100% |
| Tangential Flow Filtration | 82.1% | 91.0% |
| Affinity Chromatography | 52.5% | 77.4% |
| Size Exclusion Chromatography | 25.5% | 43.3% |
| Overall purity | 85-90% | >99% |

Fig. 7 Purification of a recombinant malaria vaccine (PFRH5) from insect cells feedstock using a C-terminal fused His6-tag or C-tag.*

- A. Purity assessment: Improved purity compared to hexa-histidine tag purification
- B. UV280 absorbance chromatograms after Size Exclusion Chromatography (left His6, right C-tag)
- C. Process yields after each purification step. C-tag clearly outperforms His6-tag (table)

*Data Obtained from: Jin, J., et al., 2017. *Accelerating the clinical development of protein-based vaccines for malaria by efficient purification using a four amino acid C-terminal 'C-tag'*. *Int J Parasitol.* 47(7), 435-446

- ✓ The first affinity tag to be left on a therapeutic protein, approved to enter clinical trials

TRADEMARKS/LICENSING

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