

Advantage of antibody based selectivity in the purification of biologics

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INTRODUCTION

Advances in biotherapeutics 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.

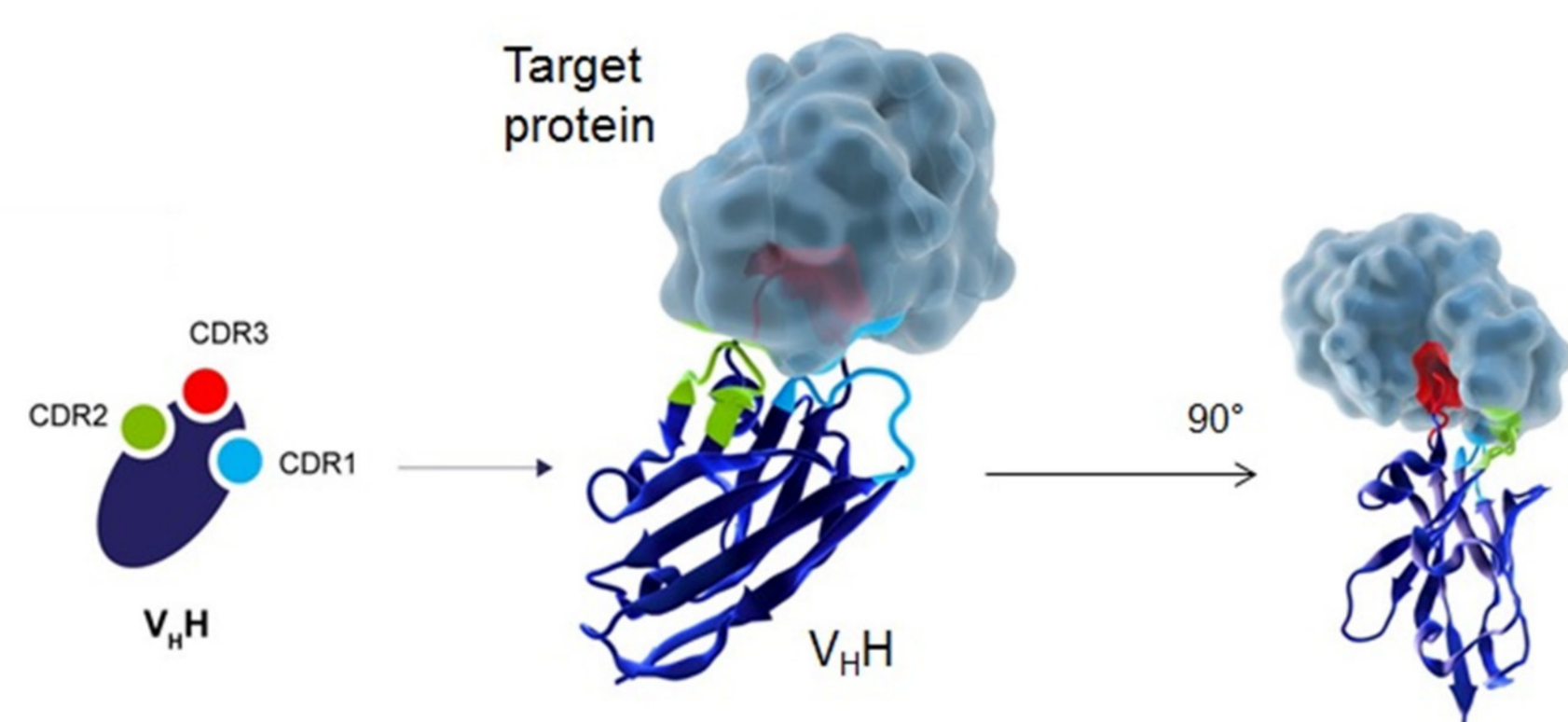
Thermo Scientific™ CaptureSelect™ affinity products and analytical tools are developed for the discovery and manufacturing of even the most demanding biotherapeutics. The affinity resins provide high target purity in a single step, independent of feedstock.

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

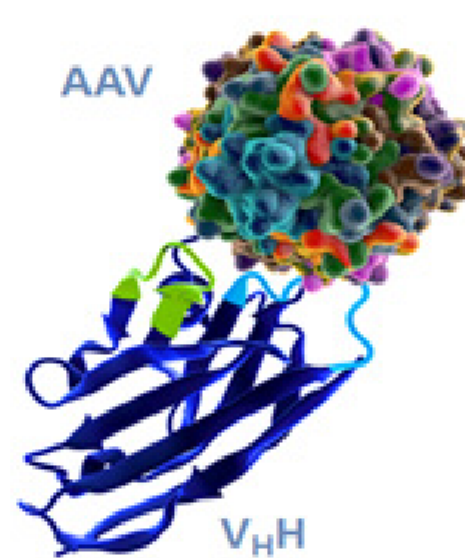
Fig.1 CaptureSelect ligands are V_HH fragments (single domain antibody fragments – sdAb), the smallest antigen binding molecule.

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.



Viral vector purification (AAV)

- Increased yields through the reduction of chromatography steps in the viral vector purification process
- High purity and recovery in a single purification step
- One chromatography platform for AAV vector purification (AAVX)



POROS™ CaptureSelect affinity resins for AAV purification

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 ^{13*}	AAV1 to AAV10, recombinant & chimeric vectors

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

AAV resin performance data

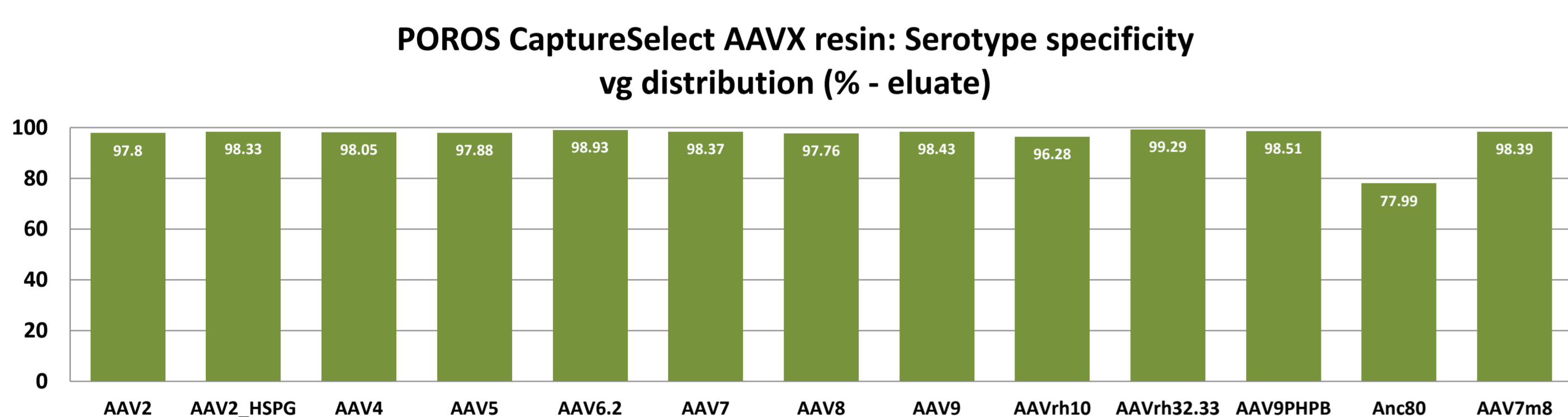


Fig. 2 To date, the AAVX ligand has shown affinity towards all serotypes tested.

The AAVX resin serotype specificity was tested using a static binding assay with a wide variety of serotypes. Resin was mixed with AAV (from clarified lysate) in a tube for 10 min. Specificity determined based on elution recovery; acidic elution buffer, pH 2 (0.1M citric acid). VG was determined by qPCR. (Data kindly provided by Massachusetts Eye and Ear)

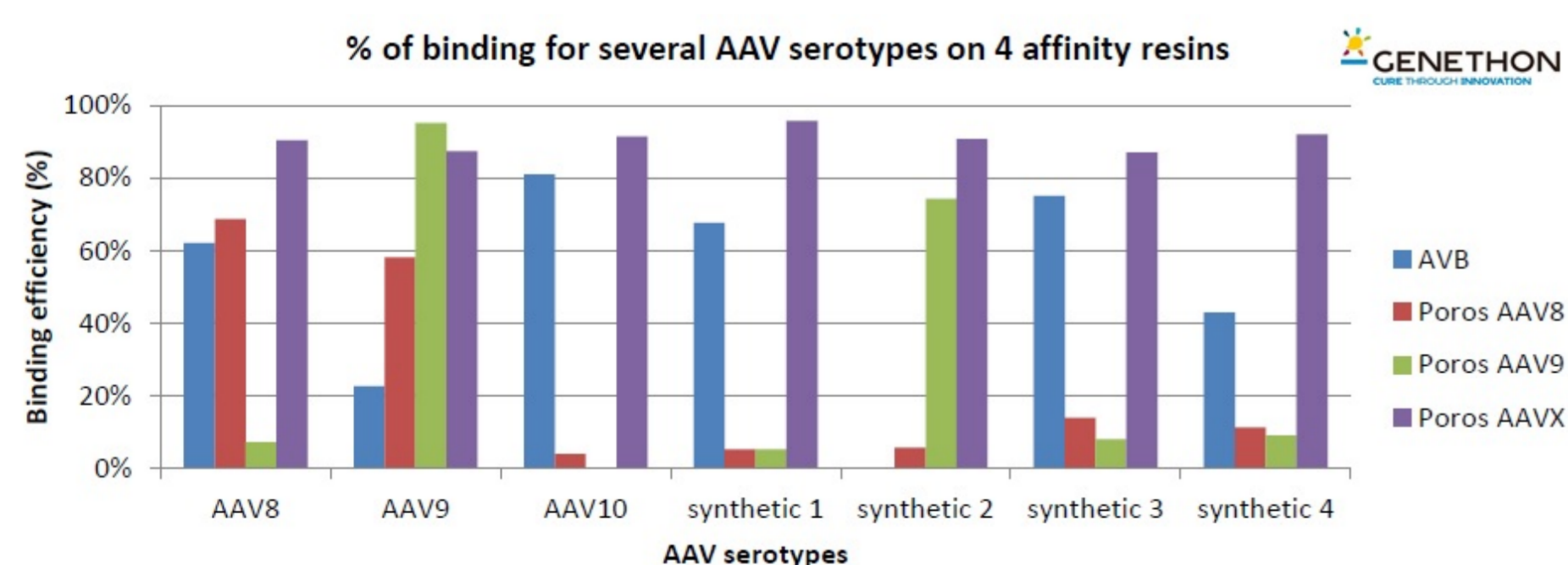


Fig. 3 Competitive analysis AAVX vs. AVB™ Sepharose

POROS CaptureSelect AAVX resin is the only resin to have high affinity binding for all these serotypes. The experiments were performed in 96 well plate format with 25µl of resin. The vectors titers were measured in the Flow Through

AVB is a trademark of GE Healthcare

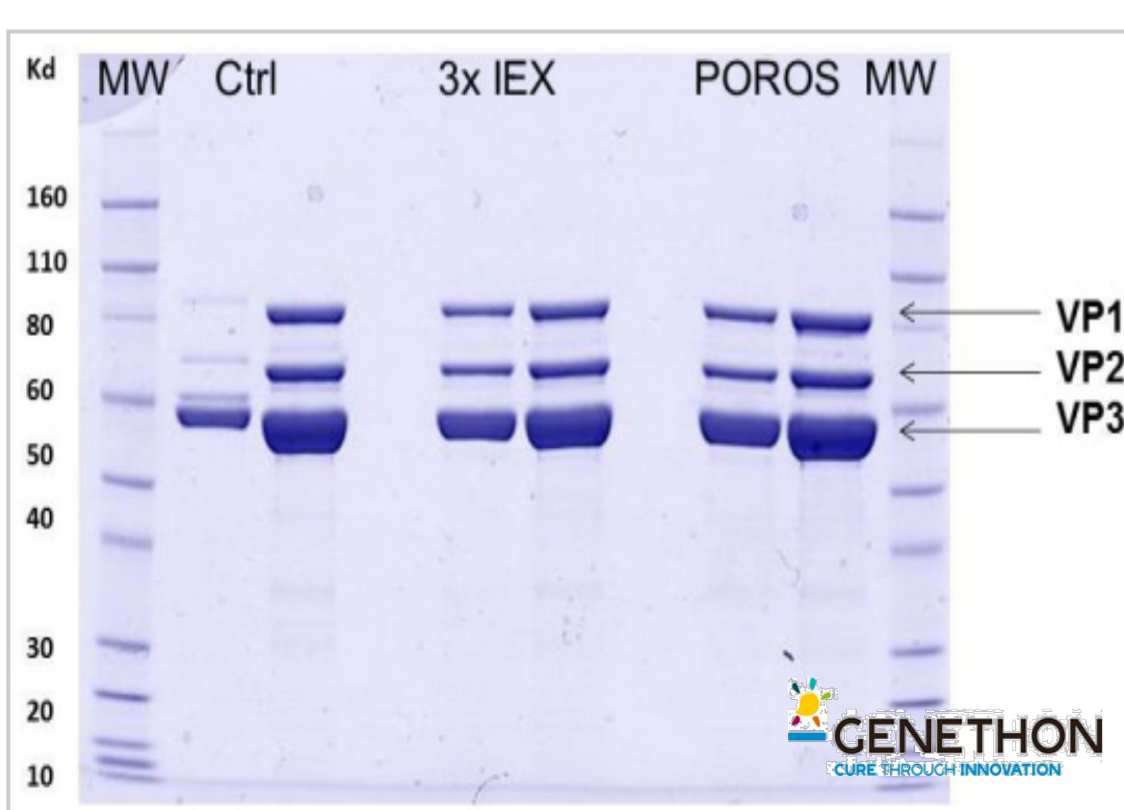


Fig. 4 High purity in a single step. POROS CaptureSelect AAV9 resin in a single step affinity process achieved an equivalent purity profile to a 3-step IEX process

- ✓ The use of AAV affinity resins simplifies processes and can reduce costs by a factor of 6 and increases process recovery from 20% to 70%

Data kindly provided by Genethon

Antibody Subdomain Targets

A unique set of CaptureSelect affinity ligands has been developed (Fig. 5), directed against a variety of antibody subdomains to help facilitate purification of a broad range of antibody formats.

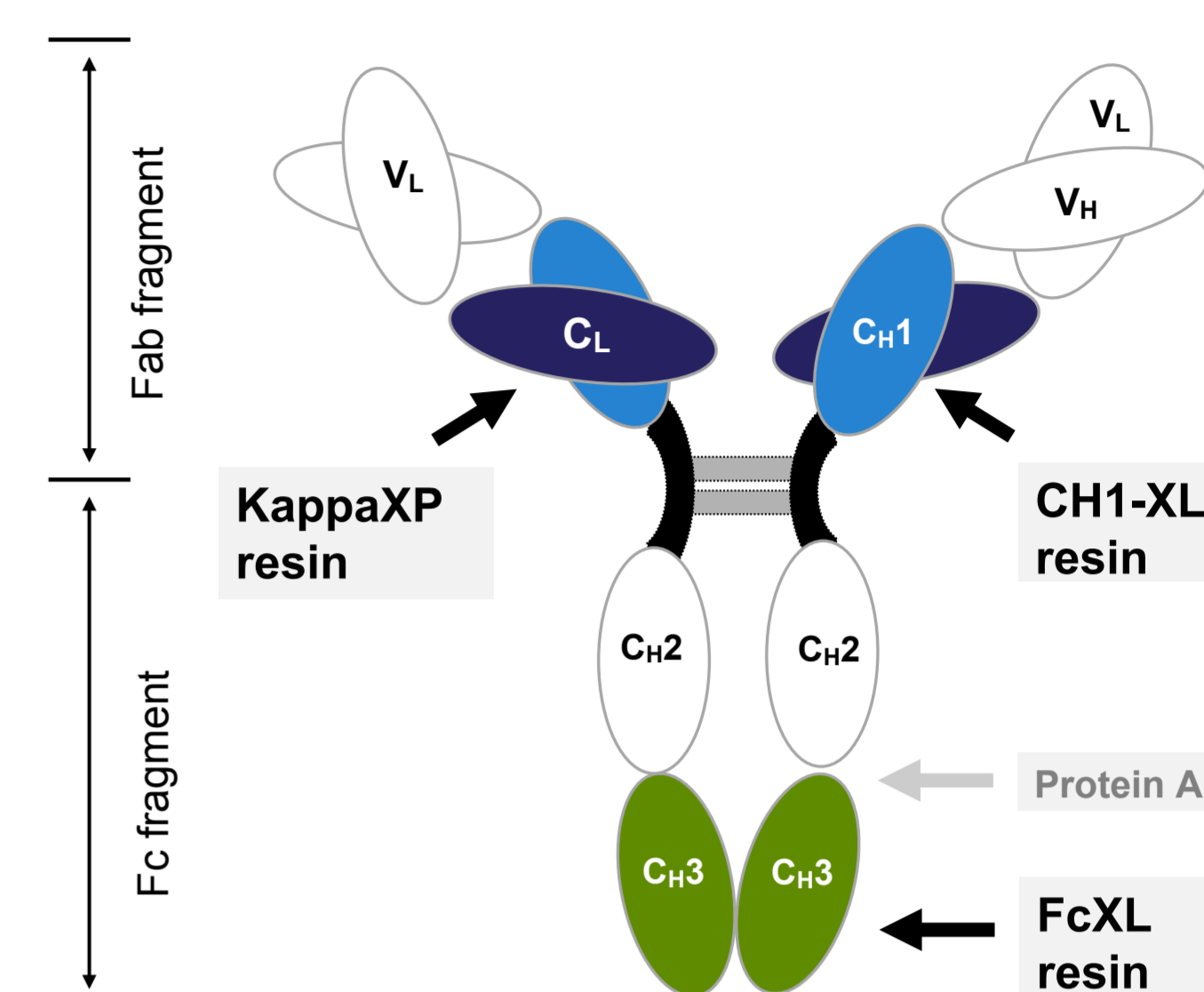


Fig. 5 CaptureSelect Antibody Selectivity Binding regions of CaptureSelect resins for affinity purification of antibodies and antibody fragments.

Antibody purification - CaptureSelect CH1-XL

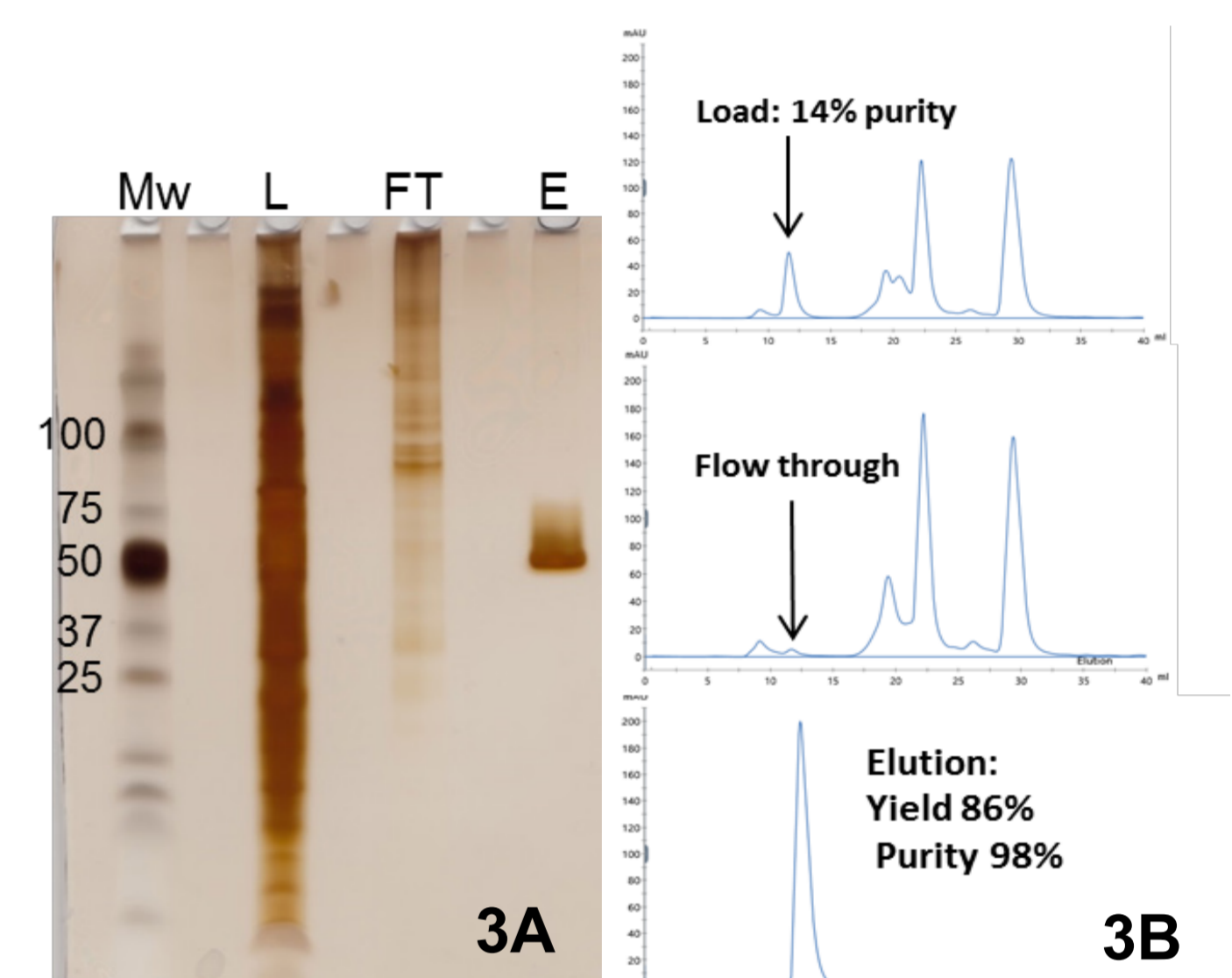
- CH1 binding domain ligand
- No co-purification of free light chains (only correct assembled Fabs)
- Efficient elution at milder pH (4 – 4.5)

✓ Fab fragment purification platform

Fig. 6 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.

3A: 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.

3B: Gel filtration analysis showing 98% purity of the Fab fragment in the elution fraction with a yield of 86%



Protein purification - CaptureSelect C-tag XL

- Unique affinity tag based on a 4 residue C-terminal peptide sequence: E-P-E-A
- Enabling high purity and yield from complex mixtures in a “one-step” process
- Mild elution, protecting the protein of interest

✓ Used in cGMP purification of a recombinant malaria vaccine

Ideal platform for scFv's, single VH and VL domains

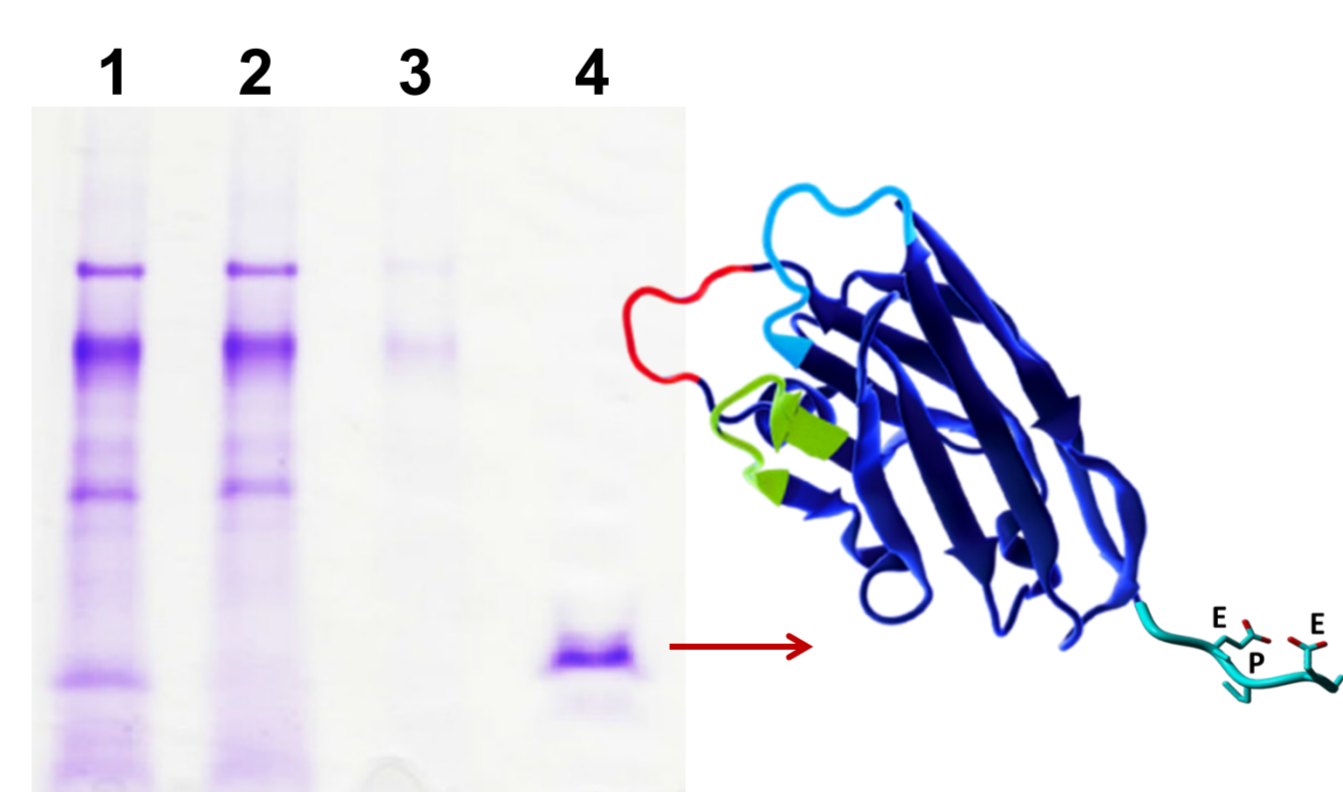
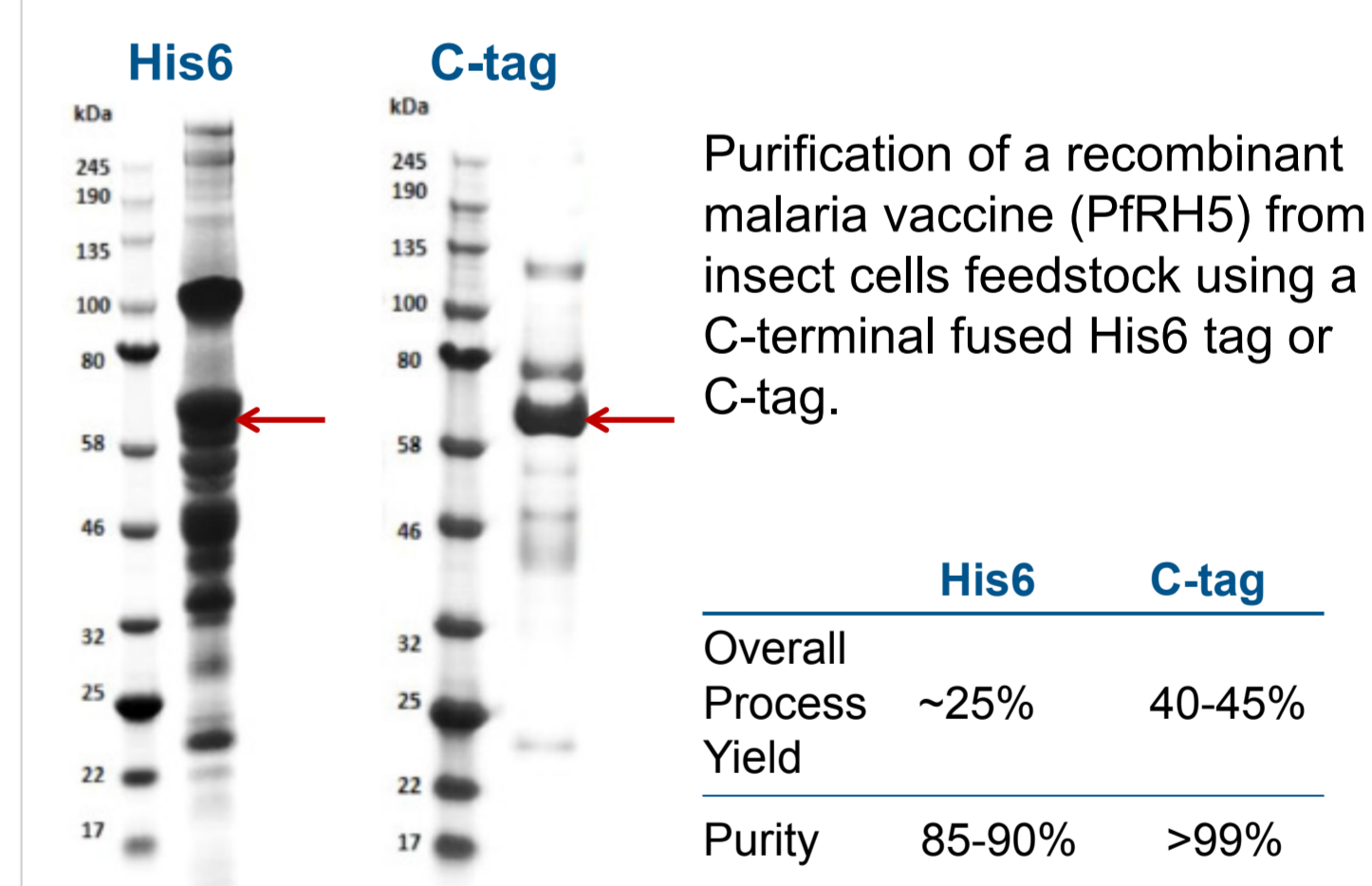


Fig. 7 SDS-PAGE analysis of the purification of a C-tagged V_HH domain

- 1 Spiked CHO cell culture harvest
- 2 Flow-through fraction
- 3 Wash (20 mM Tris, 1M NaCl, 0.05% Tw20 pH 7.5)
- 4 Elution fraction (20 mM Tris, 2.0 M MgCl₂ pH 7.5).

Improved yield and purity compared to hexa-histidine tag purification



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

CaptureSelect products and development pipeline

Product Stage	Therapeutic proteins	Viruses & Vaccines	Antibody types
Bioprocess	FSH, HSA, hCG, tPA, C-tagXL, hGH, TSH	AAV8, AAV9, AAVX, C-tagXL	KappaXP, KappaXL, FcXL, CH1-XL
Research Use Only	Antithrombin III, Fibrinogen, Transferrin, ApoH, C1-inhibitor, GM-CSF	Adenovirus 5	IgA, IgM, IgE, Bovine IgA
Lead Development	Prothrombin, DNase	Influenza (HA)	Rabbit IgG, Free LC-kappa
Lead Selection	Uni-tag, FX	Baculovirus, Lentivirus	Mouse IgG
Lead screening	IFNα/β, hIL2, FV, FXI, FXII, FXIII, FH, EPO, insulin	Exosomes	scFv, Rat IgG, IgY