Advantage of antibody based selectivity in the purification of biologics

Pim Hermans[1], Frank Detmers[1], Hendrik Adams[1], Orjana Terova[2] and Jessica de Rooij1
1. Thermo Fisher Scientific, Leiden, the Netherlands
2. Thermo Fisher Scientific, Bedford, MA

ThermoFisher SCIENTIFIC

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

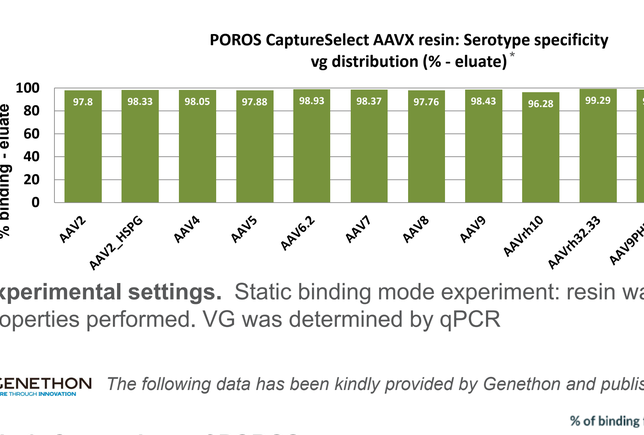
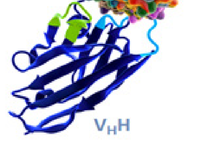


Fig.1 CaptureSelect ligands are V_HH fragments (single domain antibody fragments – sdAb), the smallest antigen binding molecules.

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 millilitre (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

POROS CaptureSelect AAVX resin: Serotype specificity vg distribution (% - eluate)*

* *Data kindly provided by Massachusetts Eye and Ear*

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

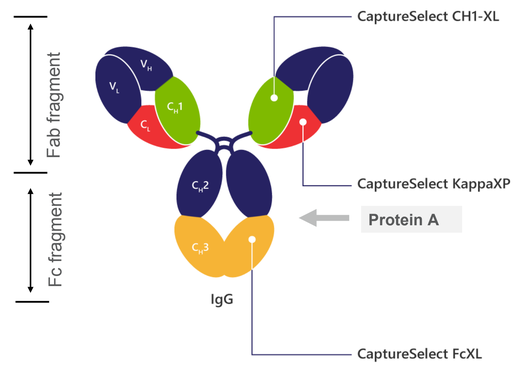
GENETHON CURE THROUGH INNOVATION *The following data has been kindly provided by Genethon and published in Cell Gene Therapy Insights* 2018; 4(7), 637-645.

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)

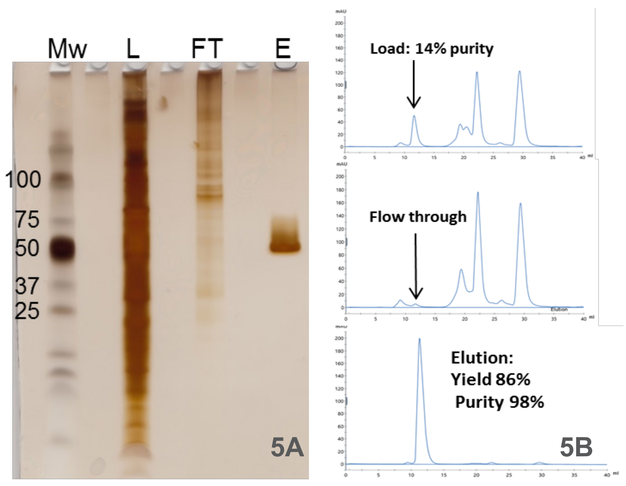


Fig.5 Ranibuzimab feed from HEK293 cells. Analysis of the fractions after purification with CaptureSelect CH1-XL resin shows high yield and purity in a single step.

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 REVOLUTIONAIRY 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

Fig 7A

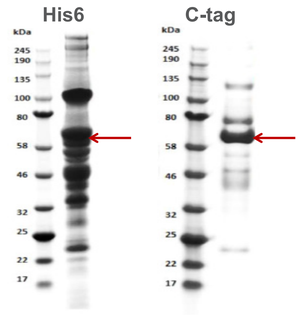
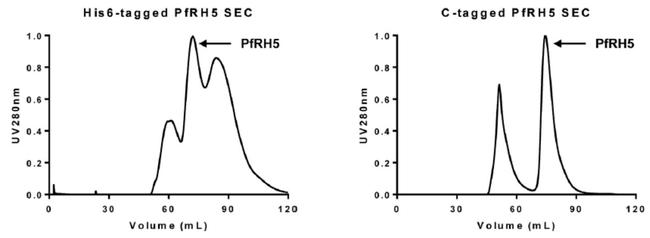


Fig. 7B



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

Learn more at thermofisher.com/captureselect

thermo scientific