Accelerating antibody drug development with subdomain-specific affinity chromatography

Pim Hermans, Frank Detmers, Kevin Sleijpen, Simon Adema, Hendrik Adams, Elina Klijs, Anja Overweel, Paul Janszen, Jessica de Rooij & Laurens Sierkstra

Thermo Fisher Scientific, Leiden, the Netherlands

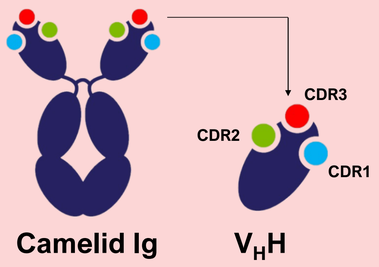
Bioprocessing

INTRODUCTION

Affinity purification platforms such as Protein A or L are well-established in the manufacturing process of therapeutic monoclonal antibodies. However, with the development of engineered modalities such as bi-specific antibodies, fragments and Fc-fusion proteins, challenges in the downstream process of these molecules arise. Affinity chromatography resins, specifically developed to bind antibody-subdomain regions, can provide an alternative solution in the purification process of these new formats. Thereby, advancing the commercial production of new antibody therapeutics.

CaptureSelect technology – unique affinity purification solution

- Technology based on single domain antibody fragments [V_HH]
- High target purity in a single step, independent of feedstock
- Unique V_HH screening technology to determine final resin properties:
  - target specificity
  - mild elution
  - ligand stability
- Scalable & animal origin free technology
- Suitable for cGMP manufacturing processes



CaptureSelect resin family for therapeutic antibody development

A unique set of CaptureSelect™ affinity resins has been developed directed against a variety of antibody subdomains, supporting manufacturers to help facilitate purification of novel antibody formats.

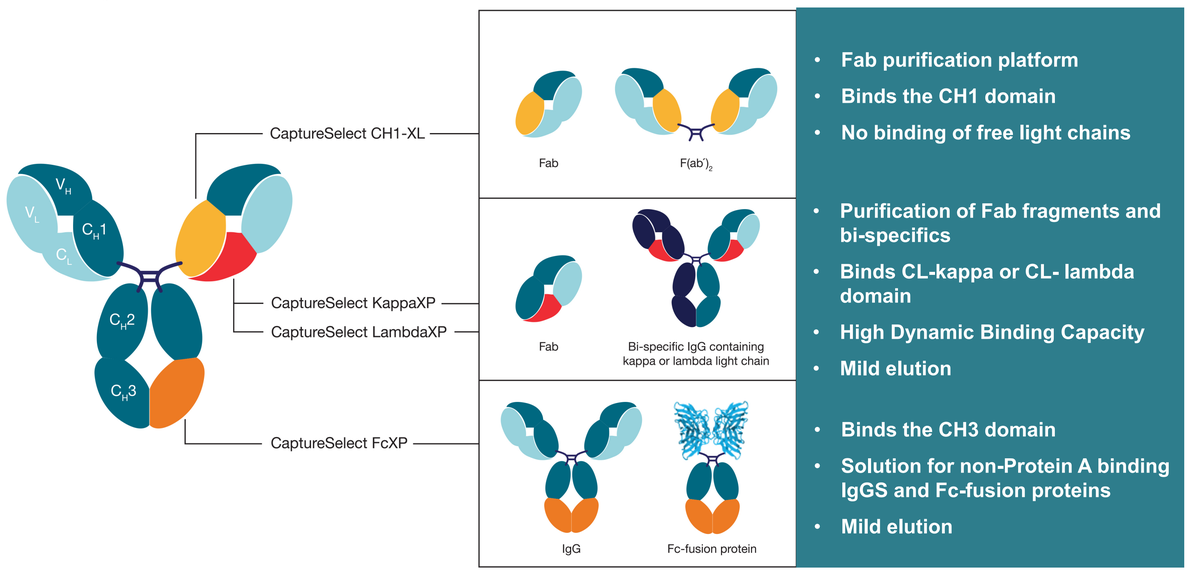


Fig.1 CaptureSelect™ Antibody Selectivity
Binding regions of CaptureSelect™ resins for affinity purification of antibodies and antibody fragments.

PURIFICATION OF ANTIBODY THERAPEUTICS - EXAMPLES

CaptureSelect™ CH1-XL affinity matrix – CH1 binding domain resin

✓ **Scalable platform solution for efficient Fab fragment purification**

- No co-purification of free light chains (only correct assembled Fabs)
- Efficient elution at milder pH (4 – 4,5)

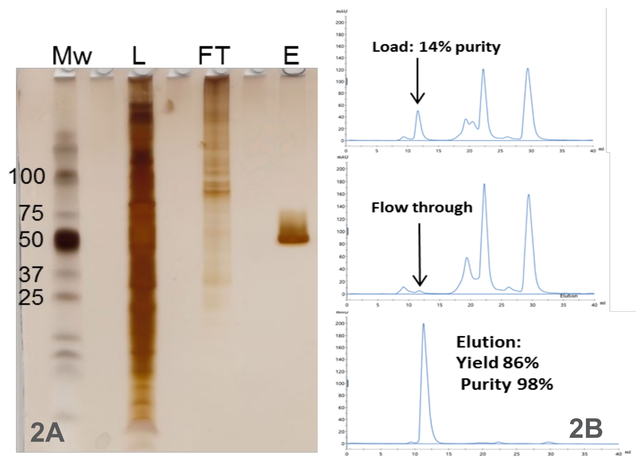


Fig.2 Ranibuzimab purification from HEK293 cells. Analysis of the fractions after purification with CaptureSelect CH1-XL resin shows high yield and purity in a single step.

2A: 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.

2B: Gel filtration analysis showing 98% purity of the Fab fragment in the elution fraction with a yield of 86%

CaptureSelect™ KappaXP & LambdaXP affinity matrices – C_L Kappa and Lambda binding domain resins

✓ **Solving purification challenges in the downstream process of bi-specific molecules**

- efficient elution at milder conditions protecting the target molecule and smaller elution pool volumes
- High dynamic binding capacity at shorter residence times

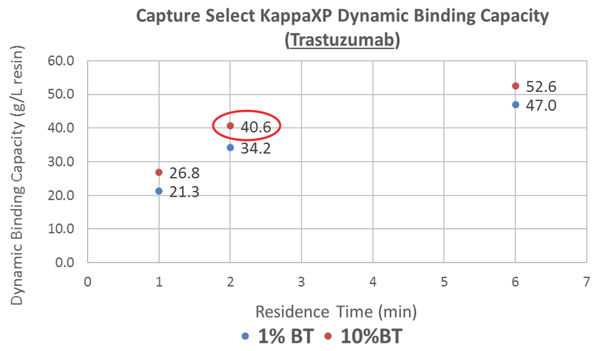
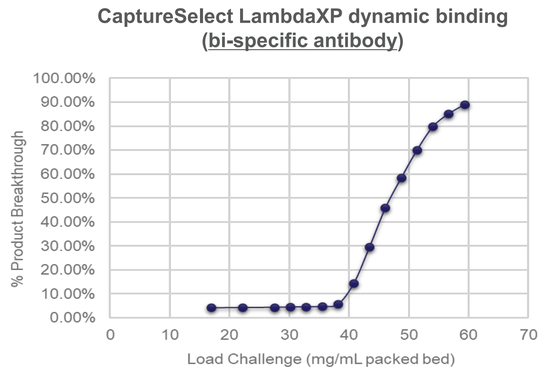


Fig.3 High dynamic binding capacity using KappaXP for Trastuzumab (humanized IgG1) purification. Dynamic binding capacity as function of residence time determined by frontal analysis.

Column format: 0.8cmDx10cm.
Feed concentration 3G/L



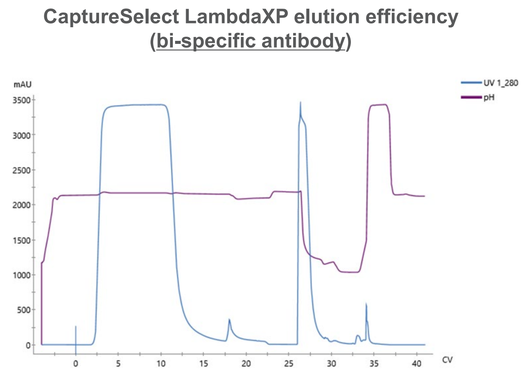


Fig.4 LambdaXP dynamic binding capacity (left) and elution (right) of a bi-specific antibody. Dynamic binding capacity at 10% breakthrough ~40 mg/mL (4 min residence time). Elution performance using 25mM sodium acetate at pH 3.6 using a load concentration of 32 mg/mL demonstrates an efficient elution of 3CVs.

Thermo Scientific resin	Dynamic Binding Capacity	Elution properties
CaptureSelect KappaXP	40 g/L at 2 min residence time	Efficient elution at milder conditions (pH 5-6) with additives
CaptureSelect LambdaXP	> 35 g/L at 4 min residence time	Efficient elution at pH 3.5-4 – small elution pool volume

CaptureSelect™ FcXP affinity matrix – CH3 binding domain resin

✓ **A purification platform for all IgG subclass molecules with an altered Protein A binding site or pH sensitivity such as Fc-fusion proteins**

- High dynamic binding capacity: >40 g/L at 4 min residence time
- Efficient elution at milder conditions (pH 4) making it suitable for Fc fusion proteins

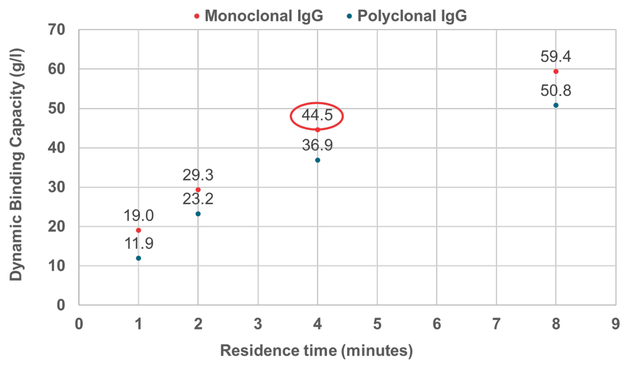


Fig.5 High dynamic binding capacity using FcXP in Rituximab and polyclonal IgG purification. Dynamic Binding Capacity as function of residence time determined by frontal analysis. Column format: 0.5cmDx20cm. Feed concentration 5 g/L Rituximab and 7 g/L polyclonal IgG.

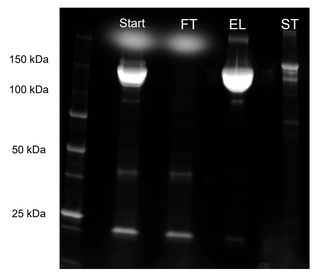


Fig.6 One-step purification from crude material with high purity. Overexpressed light chain dimers are present in the flow through (FT) but not in the elution fraction (E). ST = strip pH 2

CONCLUSIONS

CaptureSelect antibody subdomain-specific affinity resins address the purification challenges in therapeutic antibody development by providing unique selectivity, high purity and yields in a one-step purification process.

Learn more at thermofisher.com/antibody-therapeutics

thermoscientific