

Improved purification of biotherapeutics using CaptureSelect® affinity ligands



Company Overview

The Dutch biotech company BAC BV offers proprietary CaptureSelect® ligands based on highly stable and highly specific antibody fragments derived from Camelid antibody repertoire libraries. These ligands are 12-kD single domain fragments which comprise the antigen binding domain, efficiently produced by the yeast *Saccharomyces cerevisiae*.

BAC's core business area for these antibody fragments lies in the field of affinity chromatography. The ligands can be used for generic purification solutions and can be custom-tailored to solve virtually any biotherapeutic purification challenge. At the Ligand Discovery R&D department, a fast and efficient process has been developed for the discovery of new affinity ligands against a range of antibodies, plasma proteins, biosimilars and viral proteins.



Camelid antibody fragments

Classical antibodies comprise four polypeptide chains: two identical heavy chains and two identical light chains (Fig. 1A). Camelid antibodies lack light chains and the entire CH1 domain and as such has only one single variable domain (VHH) by which antigens are bound (Fig. 1A&B). The VHH domain is named CaptureSelect ligand.

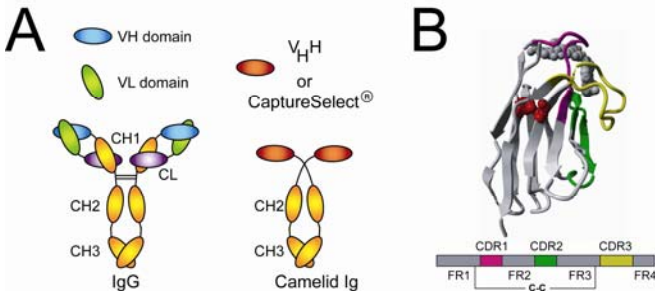


Fig. 1. (A) The difference between classical antibodies and Camelid heavy chain antibodies. (B) 3D structure of model VHH ligand R2. Note the existence of a cysteine bridge (colored in red).

CaptureSelect® Ligand Discovery

The CaptureSelect ligand discovery process consists of 3 stages (Fig. 2):

- Construction of dedicated antibody-fragment gene libraries
- Surface plasmon resonance (SPR) and ELISA is employed to analyze target binding and to evaluate ligand stability and specificity
- Ligands are expressed in yeast, followed by evaluation of their features

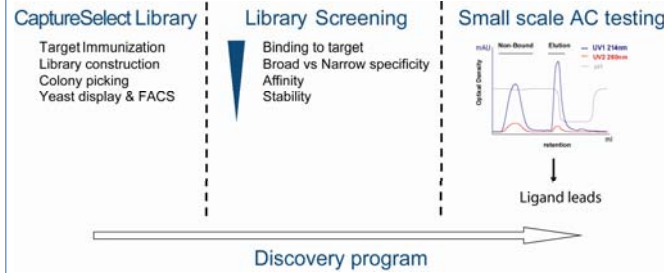
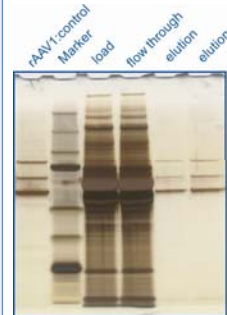


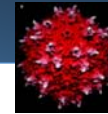
Fig. 2. The different Ligand Discovery stages.

Virus Particles: AAV

BAC has developed, together with customers, an affinity ligand for the purification of multiple serotypes of Adeno-Associated Virus. Using this ligand it was feasible to downsize a complex multi-step procedure into a high yield, high purity, 2-step purification process. The product is now widely distributed by GE Healthcare under the name "AVB Sepharose High Performance" and it is used by several end-customers for the production of clinical trial material (CTM).

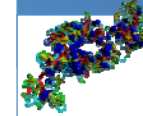


(LEFT) Purification of AAV1 using single step CaptureSelect affinity chromatography. Note that the conventional method includes density gradient centrifugation, heparin column chromatography, ion-exchange chromatography, and hydrophobic interaction chromatography.

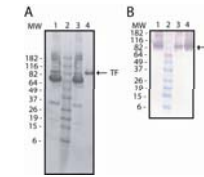


AAV2 EM photograph
Michael S. Chapman, PNAS (2002) 99: 10405

BAC's pipeline

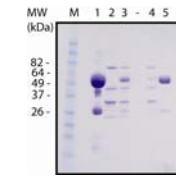
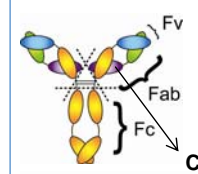


Anti-Tferrin ligands



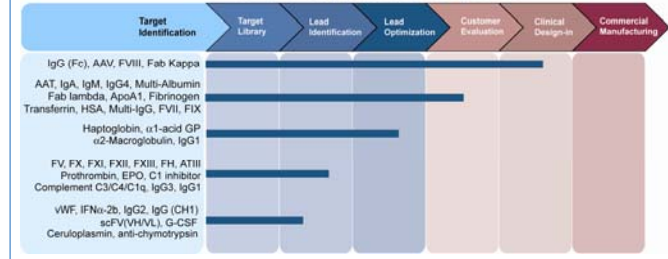
(LEFT) Typical chromatogram of a plasma protein affinity purification. Loading material: human serum 20x diluted with equilibration buffer (20 mM Tris-HCl, 150 mM NaCl, pH 7) Wash: Same as equilibration buffer. Elution: phosphate-buffered saline, pH 2. (RIGHT) Analysis of fractions. (A) Coomassie stained SDS-PAGE gel and (B) Western blotting/immunodetection with anti-TF antibodies of the fractions collected after purification. Lanes: 1, starting material; 2, molecular weight marker (kDa); 3, flowthrough; 4, Transferrin (TF) peak

The CaptureSelect Fab kappa can be used to purify human Kappa Immunoglobulins and human kappa Fab fragments in one step from human plasma. The epitope of the ligand is located at the constant domain of the kappa light chain. The product is sold under the name of KappaSelect by GEHC.



(LEFT) SDS-PAGE of samples after KappaSelect purification. Lane M, BenchMark pre-stained Protein ladder; 1, Fabs (human polyclonal Fab kappa); 2, Periplasmic fraction of *E. coli*; 3, Periplasmic fraction spiked with 0.4 mg Fab/ml; 4, Flow through fraction KappaSelect column; 5, Elution fraction KappaSelect column (0.1 M glycine pH 2.0)

BAC's CaptureSelect Pipeline



Conclusions

- BAC BV offers CaptureSelect ligands:
- ✓ for the efficient purification of any protein including viruses
 - ✓ which are produced in a completely animal component-free production process
 - ✓ at production scales ranging from 10 L, 100 L to 15 m3 in yeast
 - ✓ for use in bioprocess (sold via GEHC) for production of CTM

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