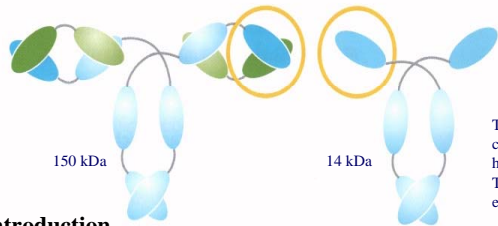


# Discovery and Implementation of Novel Affinity Ligands for Therapeutic Apheresis



The difference between classical antibodies (left) and heavy chain antibodies (right). The binding domain is encircled in yellow

## Introduction

Affinity chromatography is a well-established technology for purifying biological molecules from complex sources, like cell-culture media and plasma. These molecules are then used as (bio) pharmaceuticals. This technology can be applied in selective therapeutic apheresis when specific affinity ligands are developed. For the development of these highly selective ligands we make use of *Camelidae* heavy chain antibody fragments (Vhh), which is based on rapid identification of highly stable and specific affinity ligands against a diverse set of antigens/targets using immune *Camelidae* antibody libraries. These affinity ligands (CaptureSelect®) are then efficiently expressed in the yeast *S.cerevisiae*. The technology can be custom made to solve virtually any selective therapeutic apheresis application.

Advantages over existing methods include improved specificity, non-toxicity/immunogenicity, tuneable affinity, base stability, short development times, multiple matrices and ease of large scale non-animal derived production.

## CaptureSelect® ligands applied in IgG and endotoxin depletions

From an immune library, specific clones producing heavy chain antibody fragments were screened against Human IgG. Selected ligands were produced using the BAC's *S. cerevisiae* plug-in cloning system. The selected ligands were screened for chromatographic behaviour, the best performing ligands were further characterised for binding affinity to Human IgG subclasses and IgG from other species.

SPR Affinity	Kd
Human IgG1	5.8 * 10 <sup>-10</sup>
Human IgG2	1.7 * 10 <sup>-9</sup>
Human IgG3	4.8 * 10 <sup>-8</sup>
Human IgG4	7.9 * 10 <sup>-10</sup>

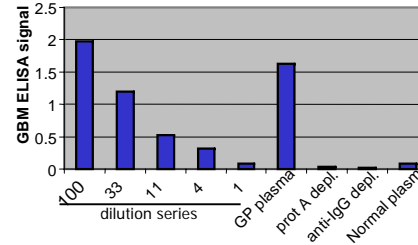
Sub class and species specificity of the ligand for Human IgG was determined using SPR on a BiaCore 3000. No cross-reactivity with Mouse IgG and Bovine IgG was found. The ligand binds all Human IgG subclasses.

Using the same technology, ligands were developed against Lipopolysaccharide L3 (LPS L3) from *Neisseria meningitidis*. The selected ligands were tested for cross reactivity with different LPS species from different Gram negative bacteria. A ligand that can bind a broad range of LPS species will be very useful in treatment for sepsis, due to an infection with Gram negative bacteria.

## Applications of CaptureSelect® Human IgG ligand in auto-immune diseases

The CaptureSelect® Human IgG ligand was immobilized on NHS-sepharose and used for the depletion of IgG from plasma from patients with Goodpasture syndrome and patients that suffered from Systemic Lupus Erythematosus (SLE). As a comparison, those samples were also depleted of IgG with Protein A sepharose. Goodpasture and SLE plasma were tested in ELISA before and after depletion and also SDS-PAGE and Immunoblots of the sera from SLE patients are shown.

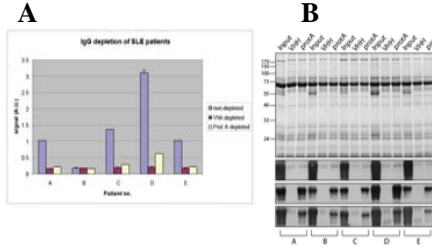
### Example 1: Goodpasture syndrome



Goodpasture plasma was tested in ELISA that detected anti-glomerular basement membrane auto-antibodies. Samples before and after IgG depletion with protein A or Human IgG ligand were tested. A dilution series of Goodpasture plasma was included to check the sensitivity of the ELISA.

A single treatment of plasma from Goodpasture patients with CaptureSelect® Human IgG ligand or Protein A results in a signal in the Goodpasture ELISA that is comparable with background level in normal plasma.

### Example 2: Systemic Lupus Erythematosus



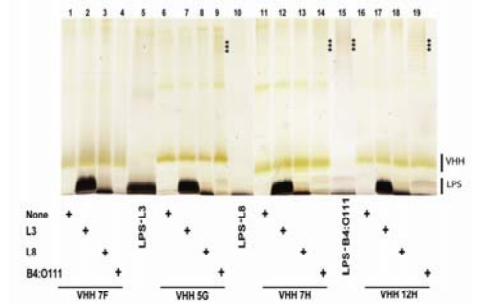
SLE plasma was tested in ELISA (A) that detected anti-nucleosome antibodies. Samples before and after IgG depletion with protein A or Human IgG ligand were tested. The same samples were analysed on SDS-PAGE and immunoblots (B) for total IgG, IgG1 and IgG3 subclass

In all SLE samples tested, a single treatment with CaptureSelect® Human IgG ligand resulted in reduction of the SLE ELISA signal to background, while treatment with Protein A in some cases (patient D and C) was not sufficient. On the Immunoblot of the serum samples from the depletion experiments is clearly shown that IgG3 is still present in the Protein A treated samples. Since auto-antibodies of the IgG3 subclass play an important role in SLE, the CaptureSelect® Human IgG ligand that recognizes all IgG subclasses performs better in the treatment of SLE sera.

## Applications of endotoxin ligand in LPS depletion

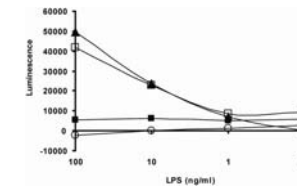
The ligand that recognizes endotoxins is able to capture LPS molecules from an aqueous solution very efficiently. It recognizes LPS molecules present in bacteria as well as free LPS molecules purified from different sources. Furthermore, it is able to detoxify LPS in whole blood. This Vhh disturbs LPS binding to their targets and disrupts its signalling that results in the generation of the effector molecules and the pathological condition of sepsis.

### Immunoprecipitation of LPS from different species



SDS-PAGE showing the amount of LPS retained by Vhh's immobilized on Talon beads. The purified LPS preparations were *N.meningitidis* L3 (lane 5) L8 (lane 10) and *E.coli* B4:O111 (lane 15). \*\*\* indicate the O-antigen containing high molecular weight LPS. Anti LPS Vhh 5G, 7H, and 12H recognize both *N.meningitidis* and *E.coli* LPS whereas anti LPS Vhh 7F only recognizes *N.meningitidis* LPS species.

### Detoxification of *N.meningitidis* L3 LPS by anti LPS Ligand



fMLP (*N*-formyl-methionyl-leucyl-phenylalanine)-induced oxidative burst was measured in whole blood after priming with *N.meningitidis* L3 LPS at the indicated concentration using chemoluminescence. 10-fold dilutions of whole blood were incubated with LPS (open squares), or with the same concentrations of LPS, which were preincubated with 100 µg/ml anti LPS Vhh 5G (filled squares), or control Vhh (filled triangles). As positive control, anti CD14 Mab 60 bca was used (open circles).

## Conclusion

*Camelidae* heavy chain antibody fragments (Vhh) can be very effectively used in selective therapeutic apheresis. Applications of Vhh's in auto-immune diseases and Sepsis are shown. The advantage of CaptureSelect® Human IgG ligand, in comparison with Protein A, is that it recognizes all IgG subclasses and is non-toxic. Our technology can be custom made and implemented for virtually any selective therapeutic apheresis application.

## Acknowledgement

This work is performed in collaboration with the Department of Cellular Architecture and Dynamics and the Department of Nephrology of the University of Utrecht, Utrecht, The Netherlands.