# Advancing VHH discovery leveraging a high throughput B cell-based platform to maximize diversity and target specificity

Sander van Duijnhoven, Sanne Meurs, Bob Berkeveld, Cynthia de Bont, Ellen van Geffen, and Ilse Roodink

*AVS Bio Netherlands B.V.*

## Introduction

The single domain antigen-binding fragments of camelid heavy chain antibodies, VHHs, combine high target specificity with an exceptionally small molecular size. Their stability, solubility, and ability to bind epitopes that are inaccessible to conventional antibodies make them powerful tools for therapeutic approaches, in particular for strategies focusing on non-traditional monoclonal IgG-based therapies. In addition, for diagnostics, their robustness and ease of recombinant production enables the rapid development of sensitive and cost-effective detection platforms, even in challenging environments.

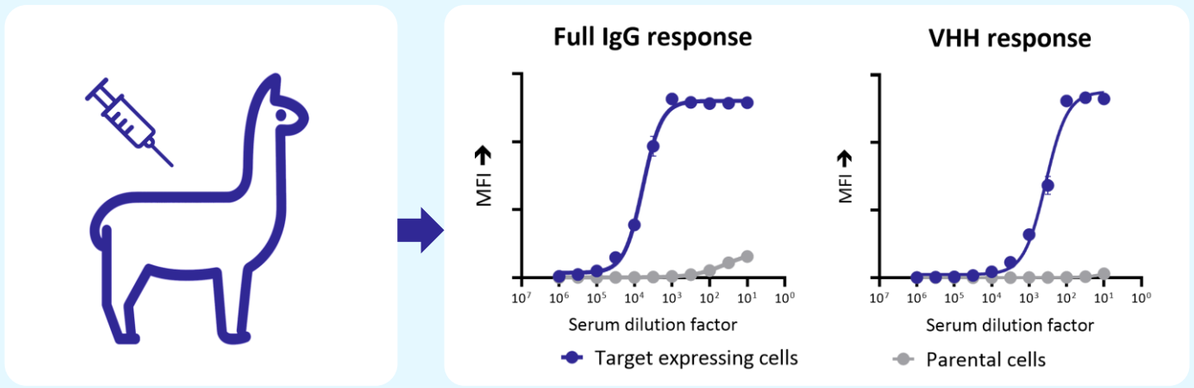
Traditionally, VHHs from immunized camelids are obtained via the well-established phage display technology. Here, AVS Bio presents a robust B cell-based, high-throughput technology that integrates direct B cell selection with next generation sequencing for v-domain recovery. The approach combines two complementary approaches: one targeting antibody-secreting plasma cells, screened in multiplex assays to identify specific target binders, and another focusing on the memory B cell population of immunized camelids. Via their surface expressed B cell receptor, memory B cells are selectively enriched for target binding prior to culture and subsequent reactivity screening. Both approaches are complemented with rapid, high-throughput recovery of VHH sequences via NGS, thereby capturing broad and distinct repertoires that together yield a highly diverse panel of candidates for subsequent lead selection.

**A robust camelid B cell platform enabling comprehensive, high-throughput reactivity and sequence profiling at a very early stage**

### Plasma B cell

- Rapid procedure
- High throughput
- Suitable for difficult target
- Multiplex screening possible
- Timeline from harvest to sequence: 4-6 weeks

### Camelid Immunization



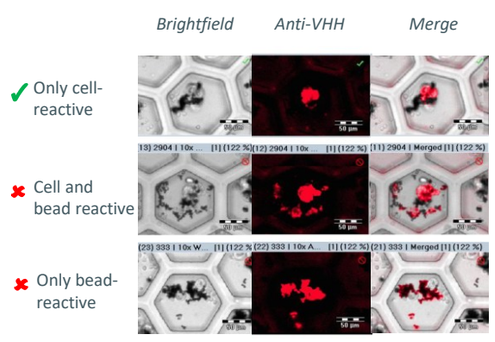

Camelids can be immunized with a variety of immunogens. For this study, llamas were immunized with recombinant proteins. Besides monitoring the immune response using conventional IgG screenings, an anti-IgG2/3 antibody is deployed to measure VHH responses before PBMC harvest.

### Memory B cell

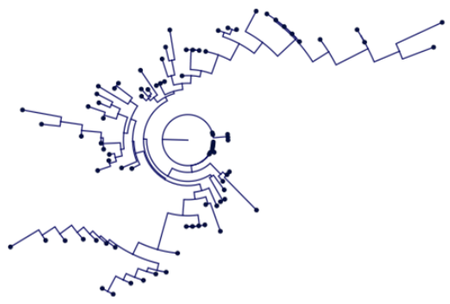
- Allows for target enrichment
- Deselection on off-targets
- Maintaining natural diversity
- Multiple screenings possible
- Timelines from harvest to sequence: 6-8 weeks

## Multiplex screening of single plasma B cells

- Co-seeding of single plasma B cells, human target-expressing cells and off-target-coated beads (human family members 2 and 3)
- Picking of only cell-reactive hits on same the day as seeding the plasma B cells. In total, 219 target specific hits (✓) were picked
- Incubation with anti-IgG2/3 to detect VHH binding
- Microscopic scanning of nanowells
- NGS-based sequence analysis revealed 67 unique VHHs with 53 unique CDR3s




Examples of nanowell selections



Phylogenetic tree of plasma B cell VHH sequences

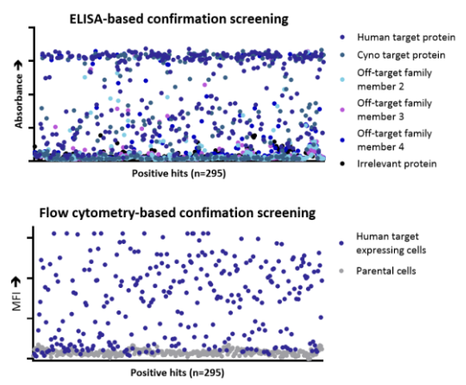
### Recovery of highly diverse VHH sequences with a high target-specificity

In total, 128 VHH-CDR3 sequences were obtained through both methods.

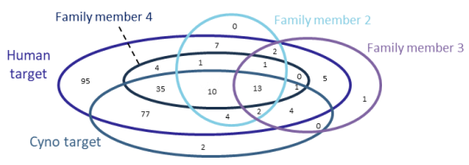
Only 2 VHH-CDR3s were overlapping, confirming both the success as well as the uniqueness of both approaches

## Multiple screenings of clonal memory B cells

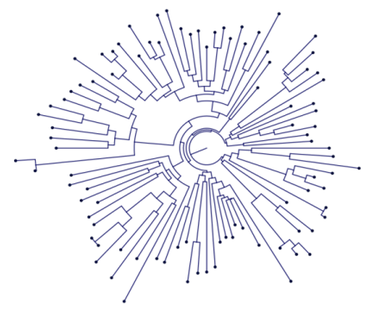
- Deselection of memory B cells population on off-targets (human family members 2&3)
- Selection on human target protein binding
- Seeding and culture of selected memory B cells in 40x 96-well plates
- ELISA-based B cell supernatant hit screening on human target protein
- Confirmation screening of hits (n=295) in ELISA on 6 targets and in a flow cytometry screening on human target expressing cells and parental cells
- Based on reactivity pattern, 90 hits were selected for NGS




Graphs depicting ELISA (top) and flow cytometry (bottom) screening results per hit.




Venn diagram showing the number of hits that were reactive towards 5 (off-)targets in ELISA. Hits with reactivity towards the irrelevant protein were omitted from this diagram.



Phylogenetic tree of memory B cell VHH sequences

- NGS-based sequence analysis revealed 91 unique VHHs with 85 unique CDR3s were obtained.

**Advanced camelid B cell selection: reducing timelines while maximizing VHH diversity and target specificity**

## Conclusion

AVS Bio optimized llama B-cell selection platform enables the rapid discovery of target-specific VHH antibodies. Multiplex binding assays facilitate simultaneous positive and negative selection, ensuring precise identification of desirable single B-cell clones. By integrating parallel selection from both plasma B cells and memory B cells, the workflow captures distinct sequence repertoires, delivering a broad and diverse pool of therapeutic antibody candidates.