

STREAMLINING VHH DISCOVERY THROUGH INTEGRATED EARLY-STAGE CLONE SCREENING BY BLI

From antigen production to high-affinity nanobody candidates

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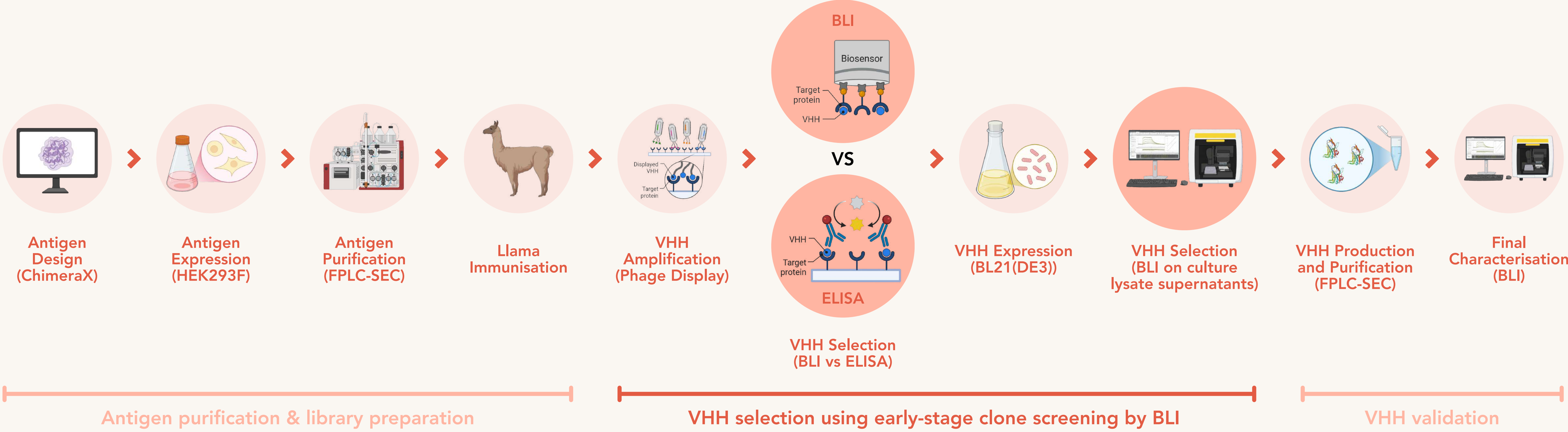
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Nanobodies (VHHs, variable domain of heavy chain of heavy chain-only antibodies) are powerful tools for medical diagnostic and therapeutic applications due to their high affinity, small size and ease of engineering. The discovery of high-quality VHH candidates requires robust, time-efficient and cost-effective workflows integrating antigen production, clone selection and biophysical characterization¹. In this work, two independent VHH discovery programs were used as case studies to establish and optimize an end-to-end VHH discovery pipeline. The Nephro program focuses on developing therapeutic nanobodies for acute and chronic kidney diseases, while the Fluosicca program aims to

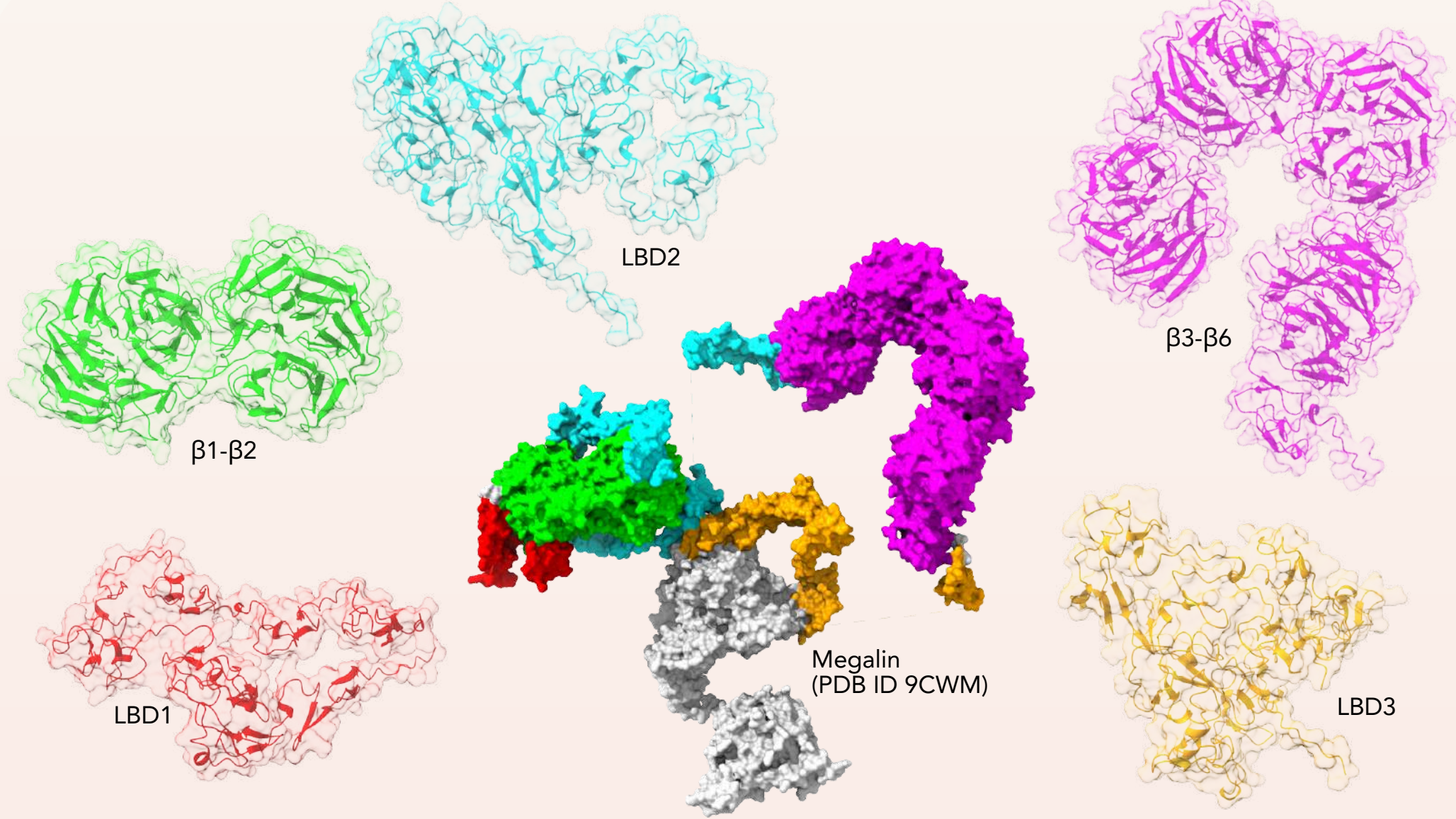
develop fluorescent VHH-based tools for diagnosis and grading of dry eye disease. Four target proteins were investigated: Megalin and Dipeptidase-1 (Nephro)^{2,3}, and HLA-DR and Lipocalin-1 (Fluosicca)⁴. A key aspect of this work was the development of a purification-free BLI (Biolayer Interferometry, Sartorius) screening strategy, allowing early assessment of VHH clones directly from bacterial lysate supernatants. This approach accelerates candidate selection, reduces downstream workload, and enables informed decision-making based on binding data from the earliest stages of the discovery process.

INTEGRATED VHH DISCOVERY WORKFLOW



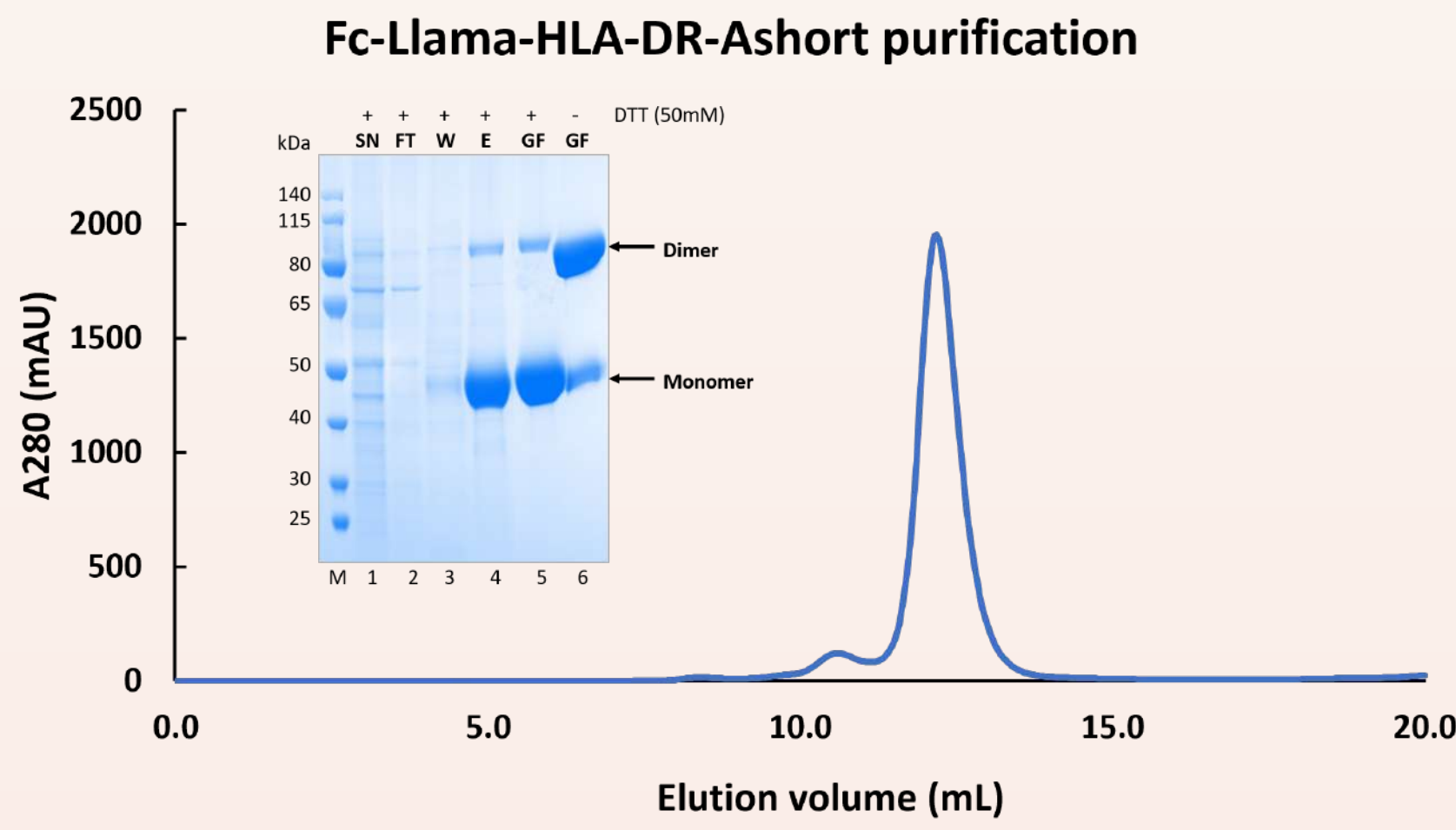
WORKFLOW IMPLEMENTATION & REPRESENTATIVE RESULTS

ANTIGEN DESIGN



Nephro project

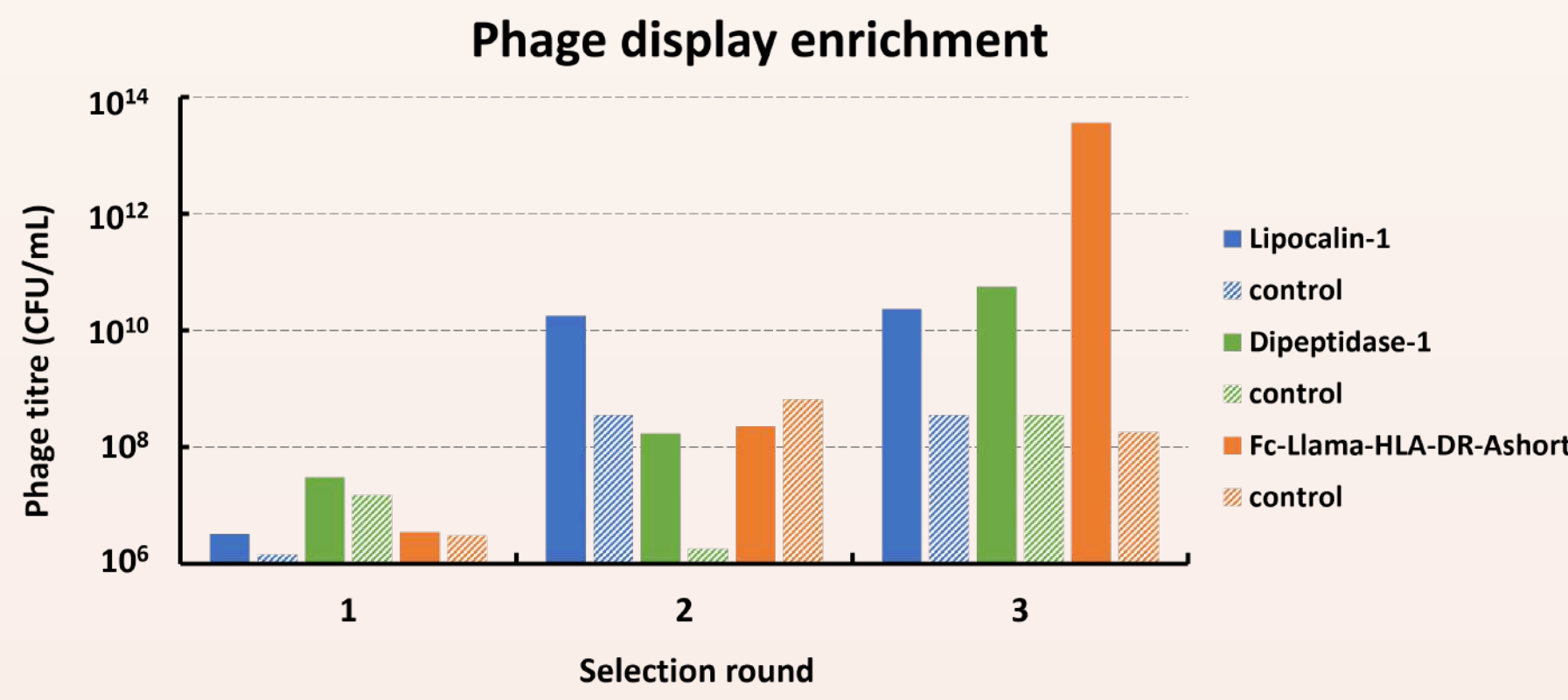
PROTEIN PURIFICATION



Fc-Llama-HLA-DR-Ashort (39 kDa monomer) was purified by Ni-NTA affinity chromatography via its N-terminal His-tag. Subsequent size-exclusion chromatography (Superdex 200 10/30) revealed a predominant monodisperse species. The elution volume (SEC) and migration pattern (SDS-PAGE) were consistent with the expected Fc-mediated dimeric assembly of the construct.

Fluosicca project

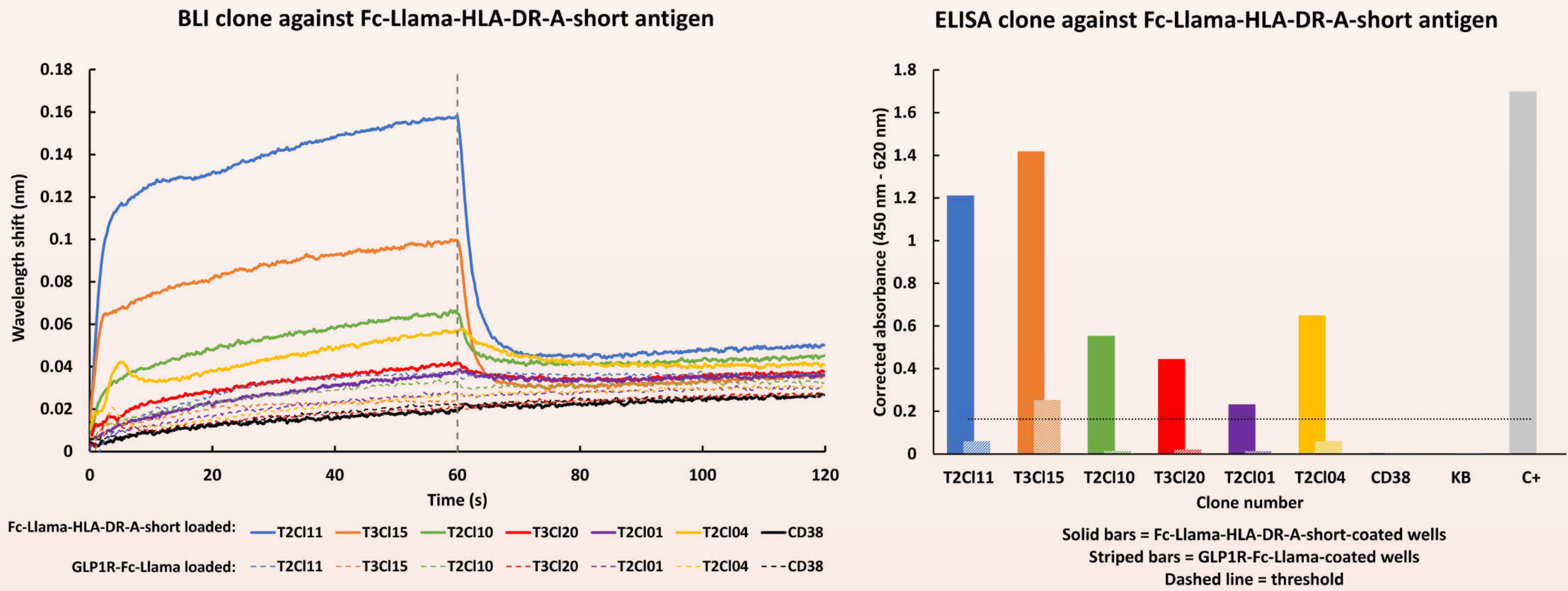
VHH ENRICHMENT



Antigen-specific VHHs against difference target proteins were enriched by biopanning through phage display. For two antigens (Lipocalin-1 and Dipeptidase-1), a ~100-fold enrichment over the negative control was achieved after two selection rounds. In contrast, a third antigen (Fc-Llama-HLA-DR-Ashort) required an additional round of selection to reach a comparable enrichment level. This may suggest a lower abundance or diversity of specific binders within the initial VHH library.

Nephro & Fluosicca projects

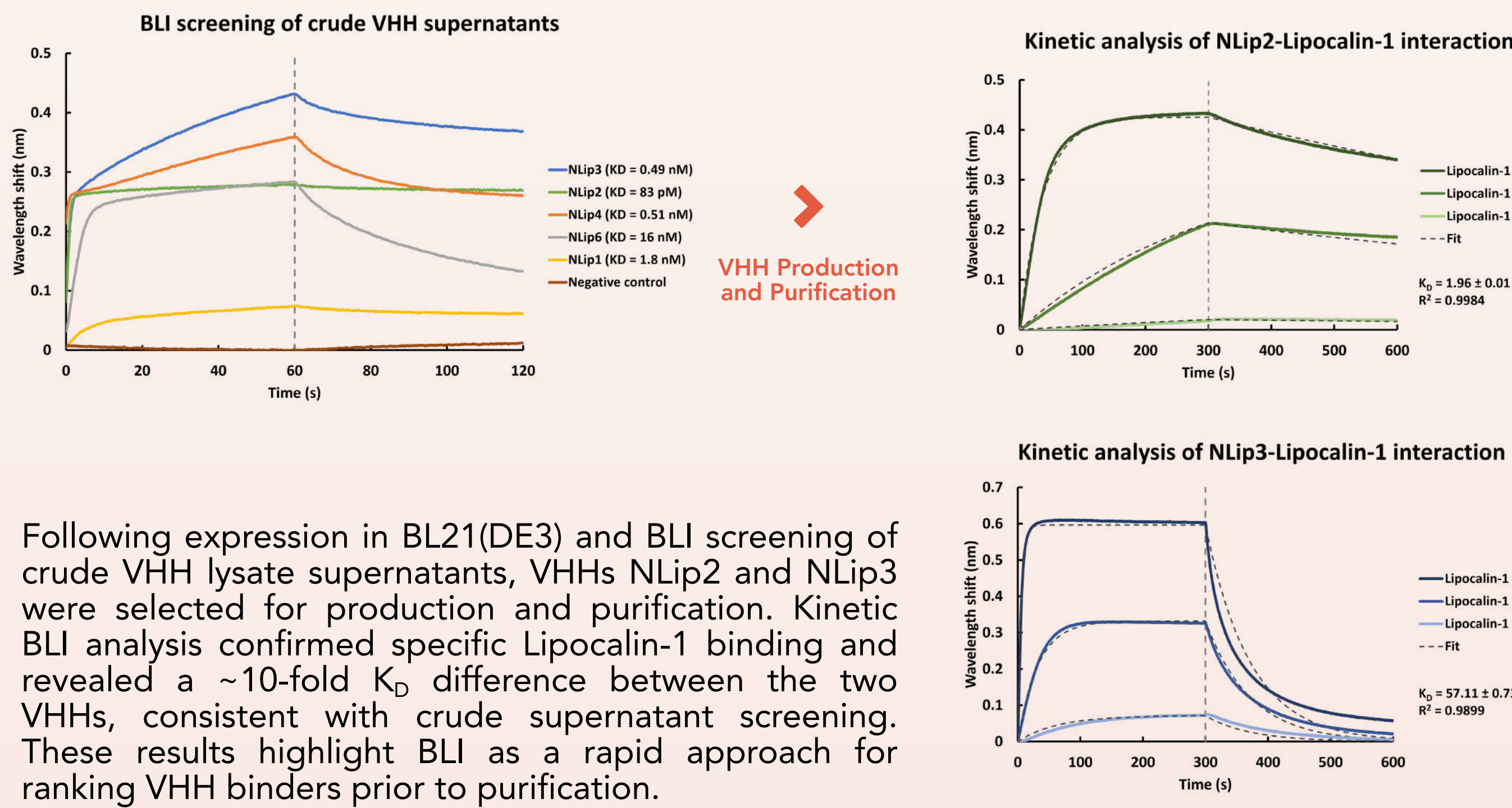
VHH SELECTION (BLI VS ELISA)



Enriched VHHs were screened by Biolayer Interferometry (BLI) and ELISA to identify antigen-specific binders. Individual phage display output clones were re-cultured, induced with IPTG and tested from culture supernatants. Target and control proteins were immobilized on ELISA plates and BLI biosensors (using biotinylated proteins for BLI measurements). Comparable binding profiles obtained by BLI and ELISA demonstrate the potential of BLI as a rapid alternative approach for identification and ranking of specific VHH binders. BLI screening enabled direct real-time detection of VHH-antigen interactions from crude culture supernatants.

Fluosicca project

VHH SELECTION FROM CULTURE LYSATE SUPERNATANTS AND FINAL VHH CHARACTERISATION



Following expression in BL21(DE3) and BLI screening of crude VHH lysate supernatants, VHHs NLIp2 and NLIp3 were selected for production and purification. Kinetic BLI analysis confirmed specific Lipocalin-1 binding and revealed a ~10-fold K_D difference between the two VHHs, consistent with crude supernatant screening. These results highlight BLI as a rapid approach for ranking VHH binders prior to purification.

Fluosicca project

OUR INNOVATION

Direct BLI screening of non-purified VHHs from bacterial culture lysates.

A rapid, label-free approach enabling real-time monitoring of antigen-VHH interactions and early binder characterisation before purification.

TAKE-HOME MESSAGES

- Rapid clone evaluation directly from crude samples
- Early kinetic information through real-time interaction monitoring
- Reduced non-specific hits compared with endpoint assays
- Lower purification workload, time and cost

REFERENCES

- ¹Desmyter et al. (2015). Camelid nanobodies: killing two birds with one stone. *Current opinion in structural biology*.
- ²Zhang et al. (2026). Receptor-mediated endocytosis by Megalin: Exploring its role in ligand interaction and disease mechanisms. *Genes & Diseases*.
- ³Whang et al. (2024). Insight into dipeptidase 1: structure, function, and mechanism in gastrointestinal cancer diseases. *Translational cancer research*.
- ⁴Blautain et al. (2024). Multimodal Approach in Dry Eye Disease Combining In Vivo Confocal Microscopy and HLA-DR Expression. *Translational vision science & technology*.