

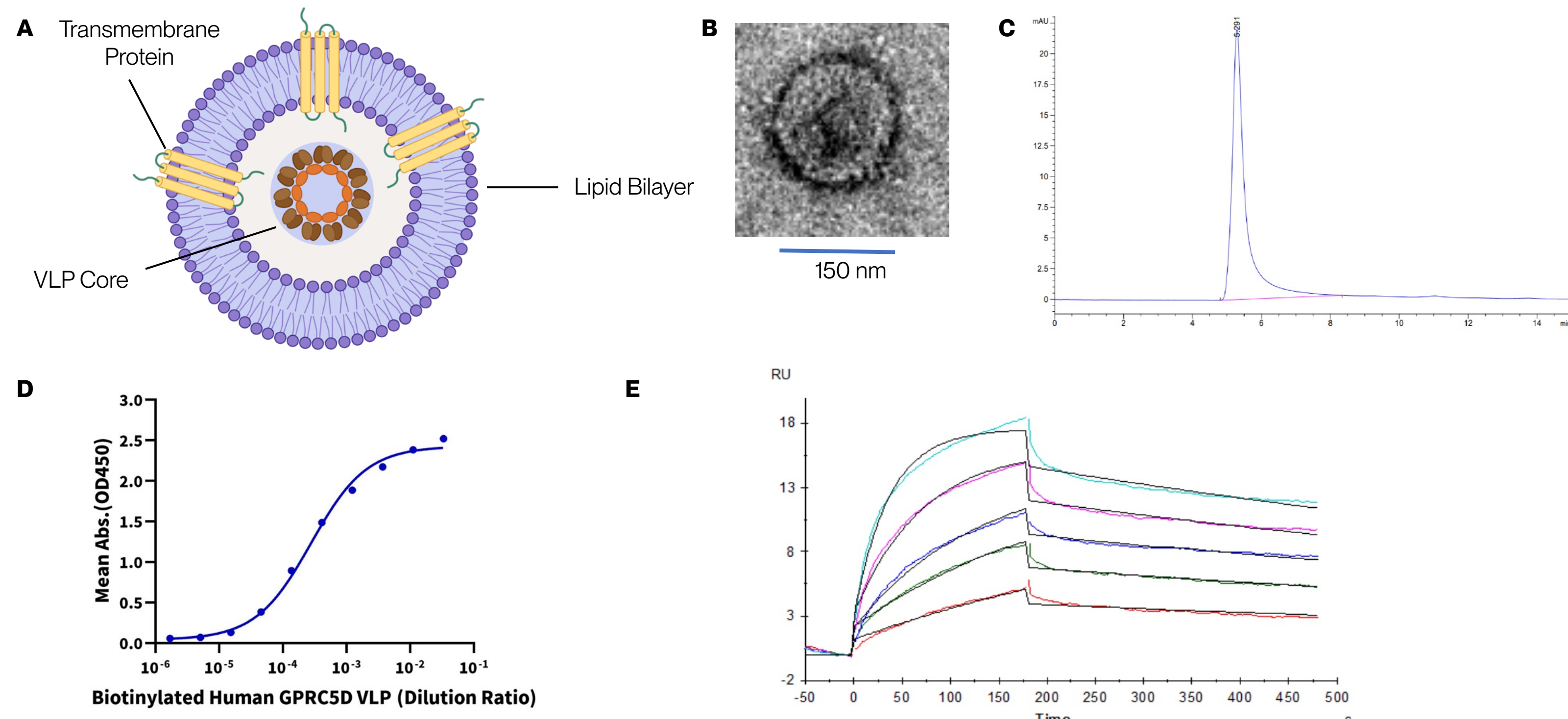


Introduction

The G protein-coupled receptors (GPCRs) protein family, also known as seven-transmembrane receptors, represents a major class of drug targets. However, extraction and purification of membrane proteins like GPCRs, are generally challenging due to low expression levels and the hydrophobic nature of transmembrane segments. To tackle this challenge, we have engineered and produced GPCRs in Virus-like Particle (VLP) and Copolymer Nanodisc formats, which have been successfully applied to GPCR antibody drug discovery and various *in vitro* assays.

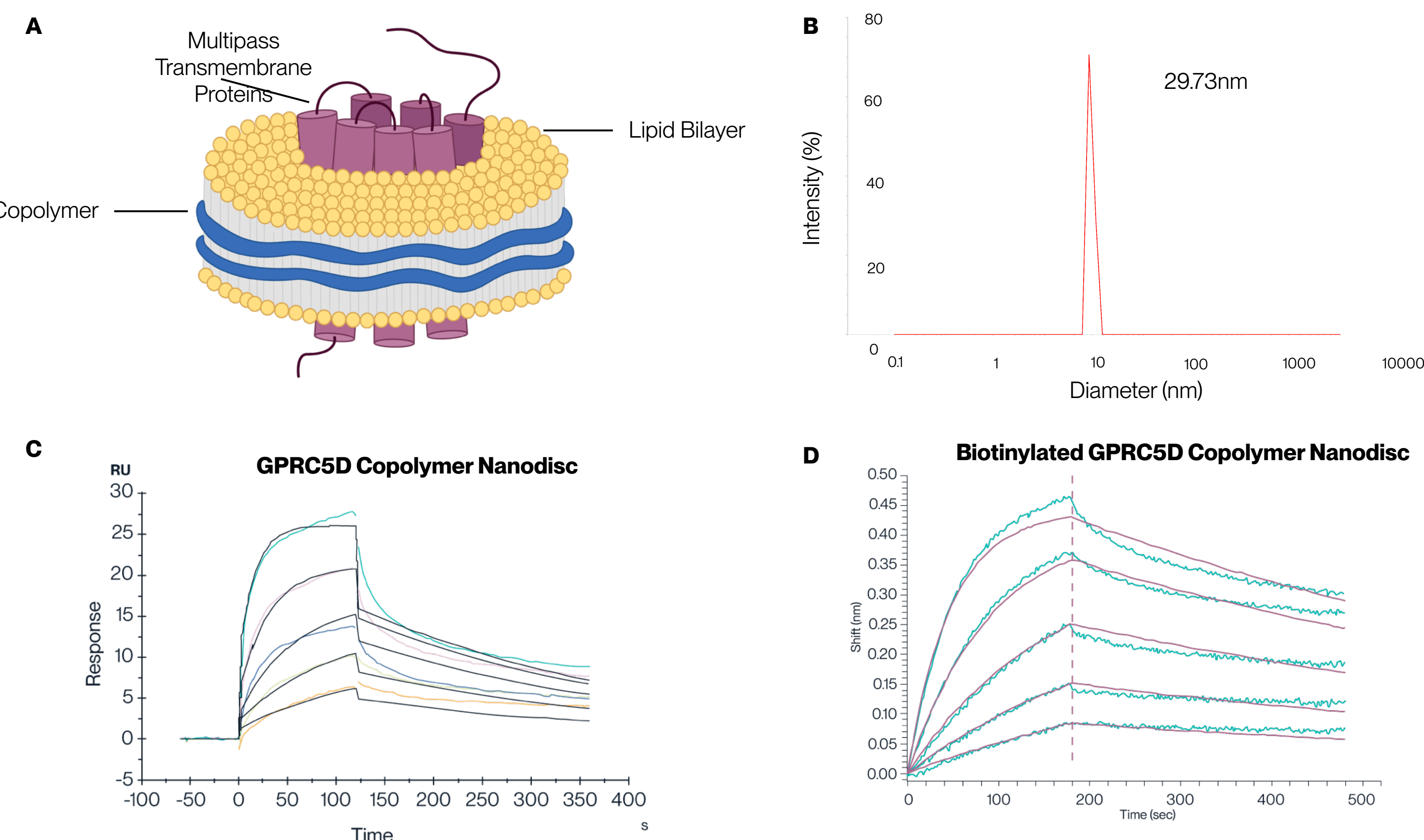
Full-length GPCRs displayed on VLPs

Virus-like particles (VLPs) are non-infectious particles that mimic the structure of viruses but do not contain genetic material. They are often as a tool of presenting multi-transmembrane proteins for various research purposes. KACTUS has successfully displayed specific GPCRs in a full-length, native conformation useful for stimulating immune responses against GPCRs.



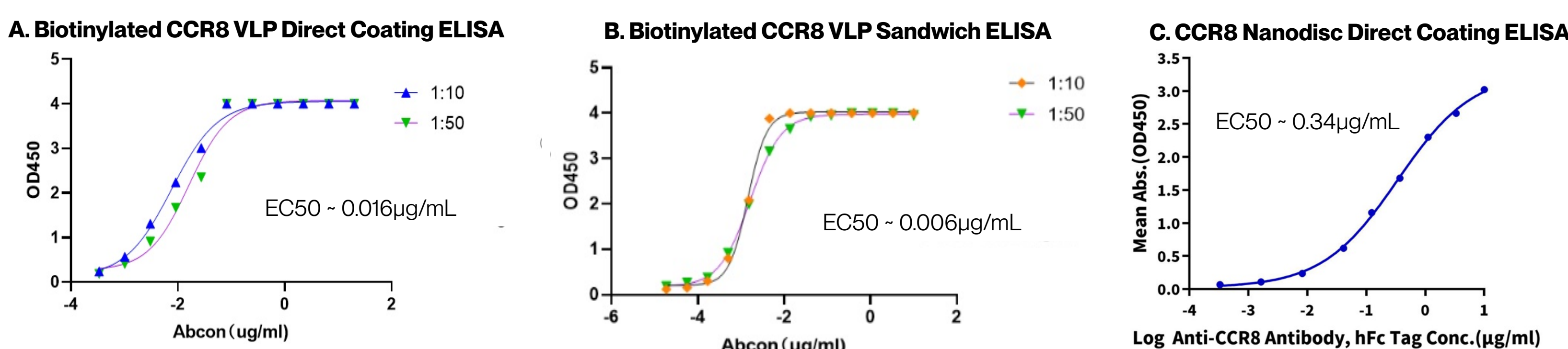
Full-length GPCRs assembled into Copolymer Nanodiscs

Nanodiscs have emerged as a powerful tool in functional and structural studies for membrane proteins. KACTUS GPCR nanodiscs are produced in a mammalian-cell based, detergent-free process. The GPCR transmembrane segments are stabilized in the center of the phospholipid bilayer surrounded by the copolymer, with intracellular and extracellular domain exposed. The assembled GPCR nanodiscs are soluble in aqueous media in a native-like bilayer environment that maintains the physiological function of GPCRs.



GPCR VLP & Nanodisc are suitable for phage panning

We tested our VLP and Nanodisc displayed GPCRs in ELISA to evaluate their potential for high-affinity interactions. Using human CCR8, we found both our VLP and Nanodisc produced robust ELISA signals, demonstrating their ability to support phage panning, a method that requires strong binding interactions for effective target enrichment.

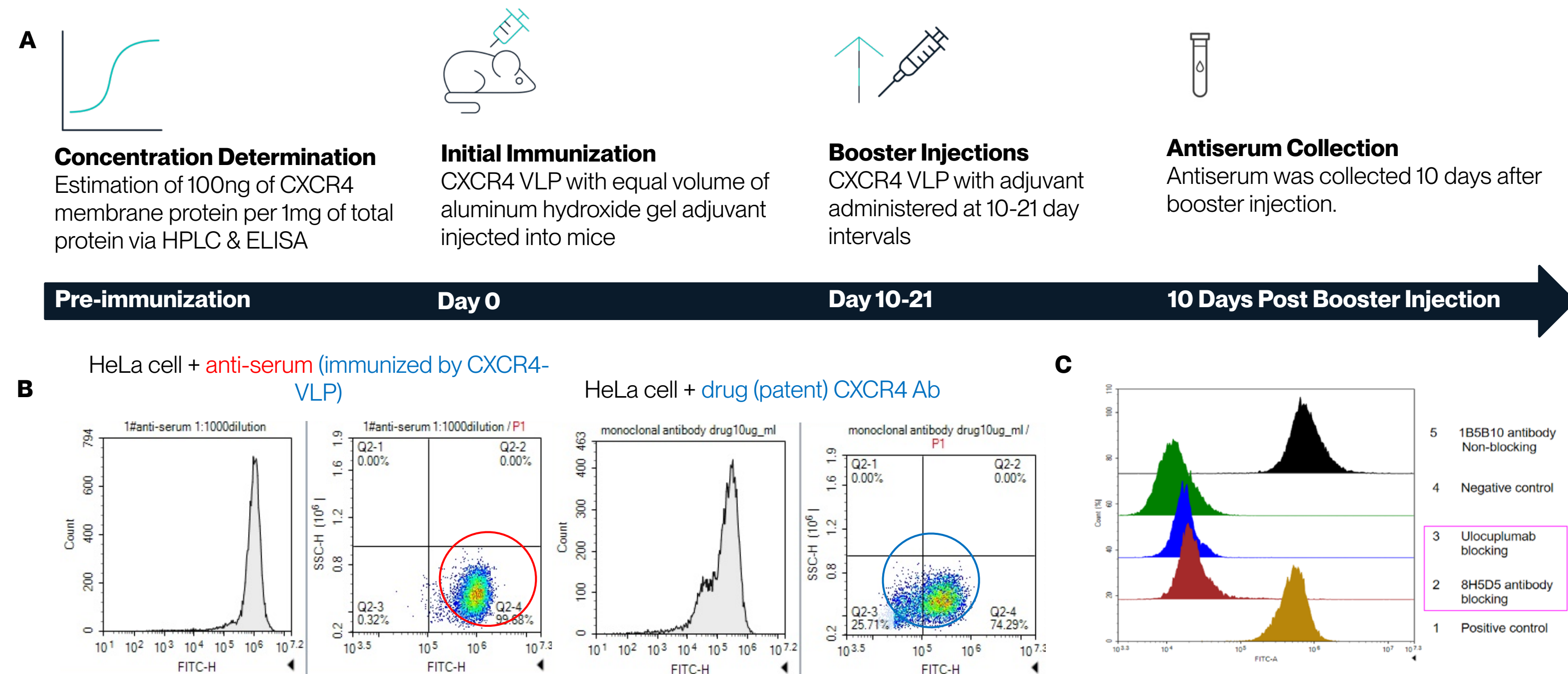


References

- Pettersen, J. M., Yang, Y., & Robinson, A. S. (2023). Advances in nanodisc platforms for membrane protein purification. Trends in Biotechnology, 41(8), 1041-1054. <https://doi.org/10.1016/j.tibtech.2023.02.006>
- Tucker, D. F., Sullivan, J. T., Mattia, K., Fisher, C. R., Barnes, T., Mabila, M. N., Wilf, R., Sulli, C., Pitts, M., Payne, R. J., Hall, M., Deng, X., Davidson, E., Willis, S. H., Doranz, B. J., Chambers, R., & Rucker, J. B. (2018). Isolation of state-dependent monoclonal antibodies against the 12-transmembrane domain glucose transporter 4 using virus-like particles. Proceedings of the National Academy of Sciences, 115(22), E4990-E4999. <https://doi.org/10.1073/pnas.1716781115>

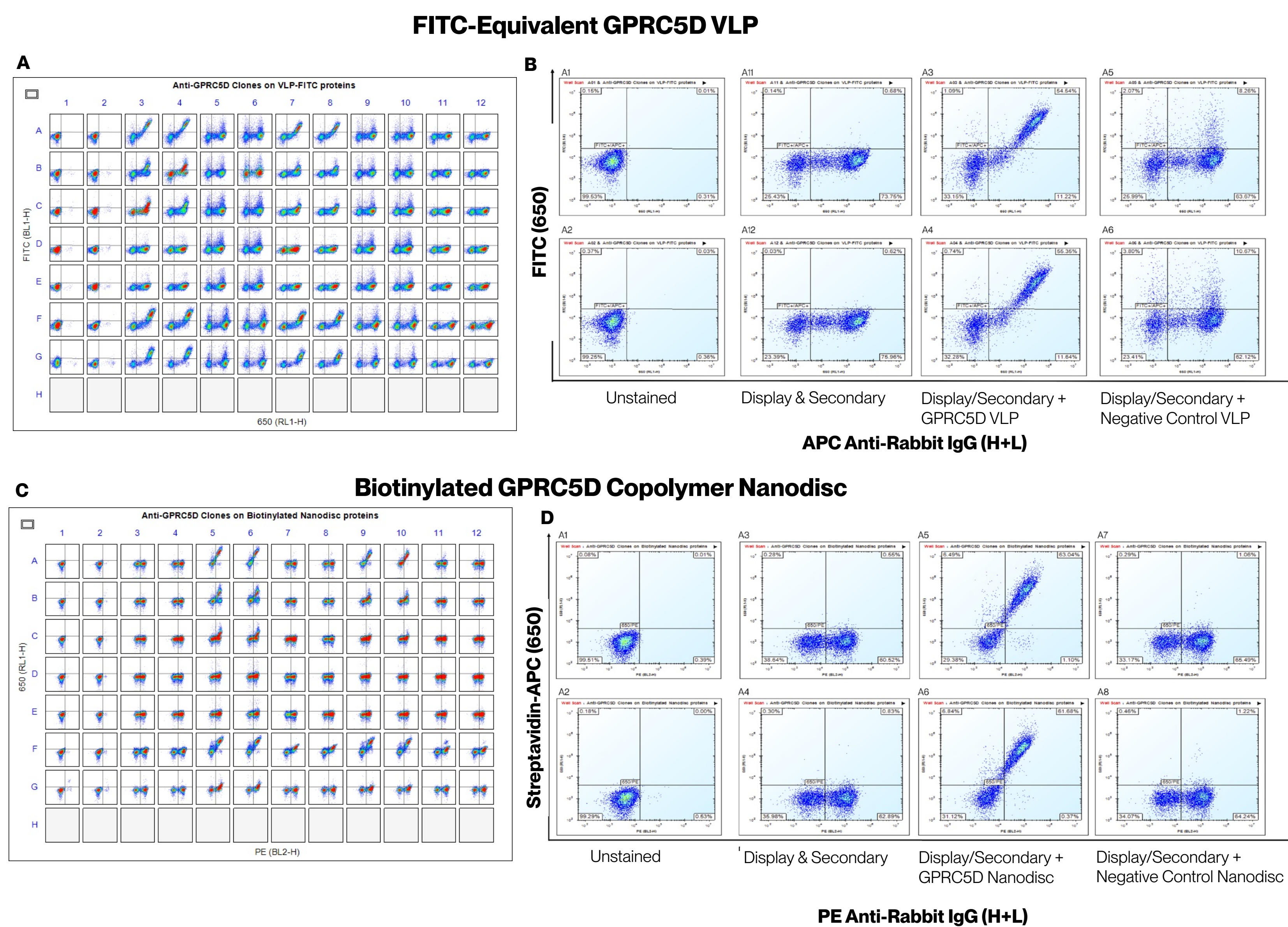
GPCR VLP immunogens yielded functional antibodies with high affinity

KACTUS initiated an in-house immunization campaign using a CXCR4 Virus-like Particle (VLP) to evaluate its effectiveness in generating high-quality antibodies. CXCR4 is a G protein-coupled receptor (GPCR) that affects leukocyte migration and is overexpressed in over 23 types of cancer, making it a prominent target for antibody development in oncology research. The goal of this study was to determine the effectiveness of CXCR4 VLP for robust antiserum generation and to functionally validate the lead antibodies through ligand blocking assays.



GPCR VLPs & Nanodiscs are effective screening targets for antibody display on yeast

A leading antibody discovery company applied our GPCR5D VLP and nanodisc to yeast display technology to evaluate the binding efficiency and specificity of anti-GPCR5D antibodies. Yeast cells were engineered to display various antigen-binding fragments (Fab) and single-chain fragment variables (scFv). The full-length GPCR5D membrane protein was provided in two different formats: FITC-equivalent VLP and Biotinylated Copolymer Nanodisc. Both formats showed robust and specific selection against positive clones.



GPCRs Produced by KACTUS

We have successfully produced or in developing the following GPCRs. To request a sample or learn more, please contact us at support@kactusbio.us.

A2AR	CCR4	CNR1	CXCR5	GLP-1R	LGR4	PAR2
APLN	CCR5	CNR2	CXCR6	GPR56	LGR5	S1PR5
BILF1	CCR6	CRTH2/PTGDR2	CXCR7	GPR75	LGR6	SMO
C5AR1	CCR7	CX3CR1	DRD1	GPR81	LPAR1	SSTR2
C5AR2/GPR77	CCR8	CXCR1	EDNRA	GPR84	MRGPRX2	SSTR4
CB1	CCR9	CXCR2	EDNRB	GPR87	NPSR1/GPR154	Steap-1
CB2	CCR10	CXCR3	GCCR	GPRC5D	OPRM1	
CCR2b	CGRPR/RAMP1	CXCR4	GIPR	LGR3	PAR1	

Conclusion

- KACTUS proprietary membrane proteins in VLP and/or copolymer nanodisc formats are powerful tools for antibody discovery research and bioanalytical assays development and validation.
- Our GPCR VLPs with crucial post-translational modifications are effective immunogens for antibody discovery campaigns, as shown in our CXCR4 VLP immunization.
- Our GPCR VLPs and nanodiscs have been validated applicable for yeast panning, with solid potential for phage panning as well.

Acknowledgement

We would like to thank GatorBio (www.gatorbio.com) for their invaluable support of performing BLI assays on nanodiscs and sharing the data with KACTUS throughout the research.