Harnessing VLPs & Nanodiscs to Unlock Antibody Discovery for Challenging Membrane Proteins

Zhi Cheng[1], Zhaojie Feng[1], Shicheng Chen[1], Tingxu Chen[1] , Jia Cui[1], Huaiyu He[1], Yuting Shi[1], Xiaoyuan Ran[1], Man-Hee Suh[1]

[1]Kactus Bio Inc.

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Introduction

Multi-pass transmembrane proteins (MP-TMPs) such as G protein-coupled receptors (GPCRs), ion channels, and transporters, play essential roles in maintaining normal physiological functions including signal transduction, cellular communication, and tissue homeostasis. Mutations in these proteins are frequently implicated in a wide range of diseases, making them highly attractive and validated targets for therapeutic antibody discovery.

However, producing stable and functionally active MP-TMPs remains a major bottleneck in drug research and development. Their reliance on a membrane environment for correct folding and activity poses significant challenges for recombinant expression and structural analysis. To address this unmet need, we leverage virus-like particles (VLPs) and nanodiscs as innovative platforms for the functional display of MP-TMPs in their native conformation. These systems provide native lipid bilayers that stabilize target proteins and enable downstream applications such as animal immunization, antibody screening, and analytical method development. Our approach offers a robust and scalable solution to accelerate antibody drug discovery against this critical class of targets.

Full-length STEAP1 proteins 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 used as a tool of presenting multi-transmembrane proteins for various research purposes. KACTUS has successfully displayed STEAP1, an emerging new drug target for prostate cancer, as well as other types of multi-pass transmembrane proteins in a full-length, native conformation useful for boosting immune responses.

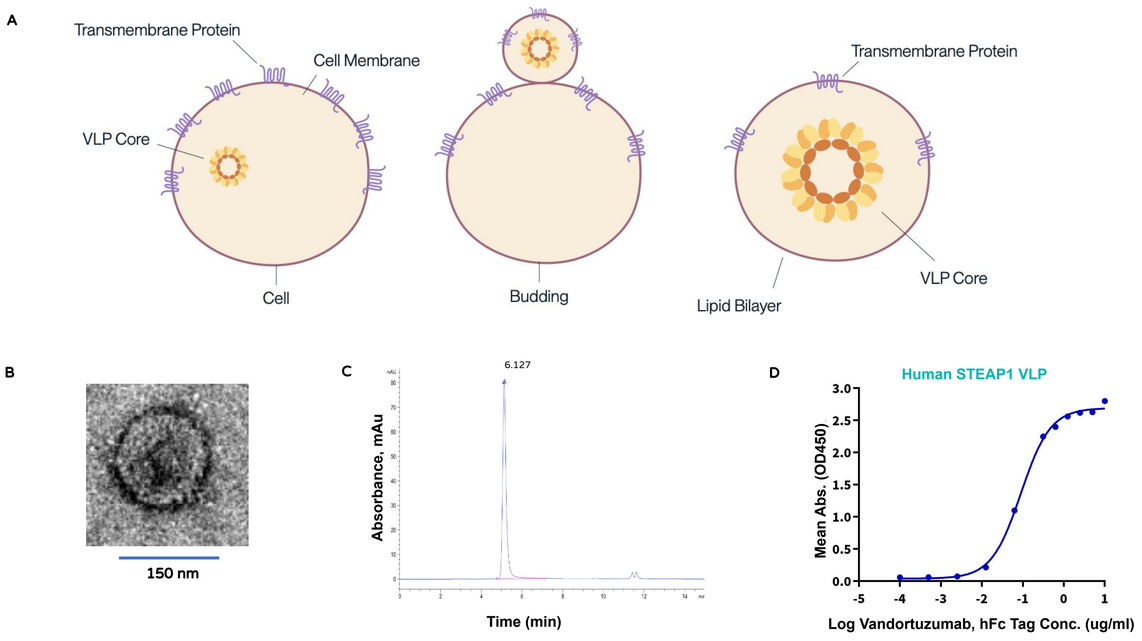

Figure 1. **(A)** Schematic diagram showcasing the budding process of VLPs from HEK cells. **(B)** VLPs are approximately 150 nm in diameter, imaged using transmission electron microscope (TEM). **(C)** The particle size of Human STEAP1 VLP is homogeneous and the particle purity is greater than 95%. **(D)** Immobilized Human STEAP1 VLP at 5ug/ml (100ul/well). Dose response curve for Vandortuzumab with the EC50 of 89.8 ng/ml determined by ELISA.

Full-length A2AR assembled into copolymer Nanodiscs

Nanodiscs have emerged as a powerful tool in functional and structural studies for membrane proteins. KACTUS nanodiscs are produced using SMA in a mammalian-cell-based, detergent-free process. The transmembrane segments are stabilized in the center of the phospholipid bilayer, surrounded by SMA, with intracellular and extracellular domains exposed. ARAR, a critical GPCR target for solid tumor indications, is soluble in nanodisc format, a native-like bilayer environment that maintains the physiological function of GPCRs and other transmembrane proteins.

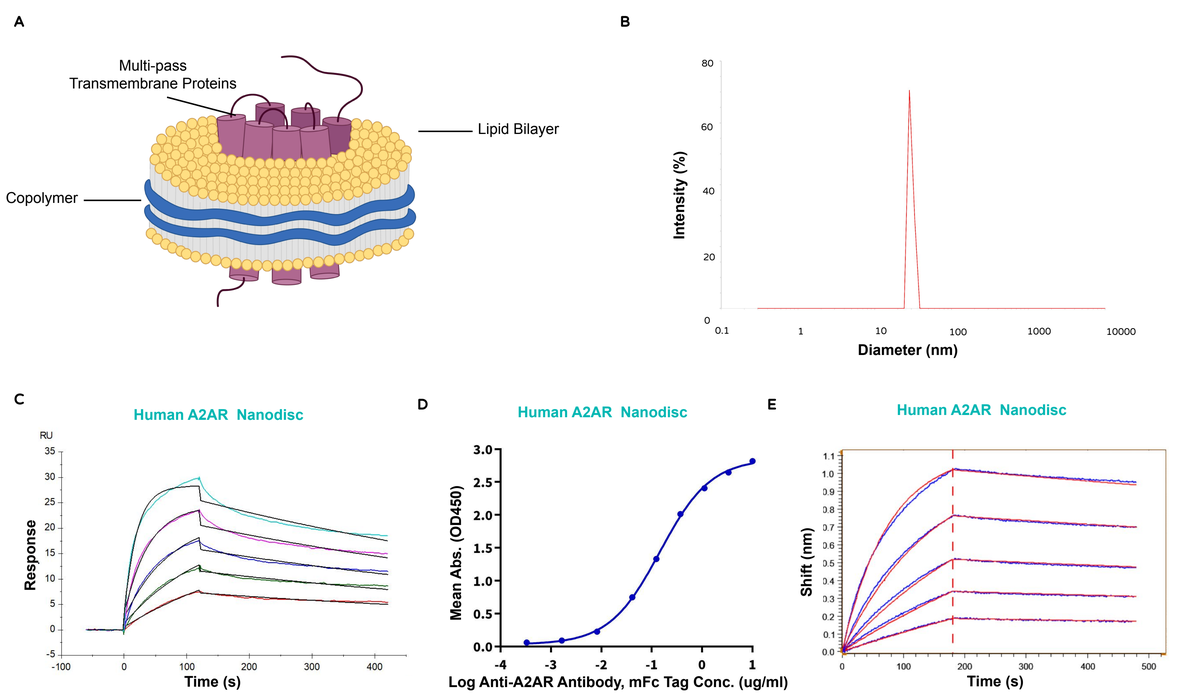

Figure 2. **(A)** Schematic diagram of GPCR assembled into a copolymer nanodisc. **(B)** The average diameter of Nanodiscs is 29.8 nm as measured by DLS. **(C)** SPR analysis of Human A2AR Nanodiscs binding against anti-A2AR mAb (Affinity Constant 0.32nM) **(D)** Immobilized Human A2AR nanodisc at 2ug/ml (100ul/well). Dose response curve for Anti-A2AR Antibody with the EC50 of 0.15ug/ml determined by ELISA. **(E)** BLI (GatorBio) analysis of Human A2AR Nanodiscs binding against anti-A2AR mAb (K_D= 4.44nM).

References & Acknowledgements

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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 their research.

VLP-displayed 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 robust antiserum generation and to functionally validate the lead antibodies through ligand blocking assays. CXCR4 is a G protein-coupled receptor (GPCR) that plays a significant role in cell signaling, leukocyte migration, and development. CXCR4 is involved in a series of oncological and autoimmune diseases, making it a prominent target for antibody development.

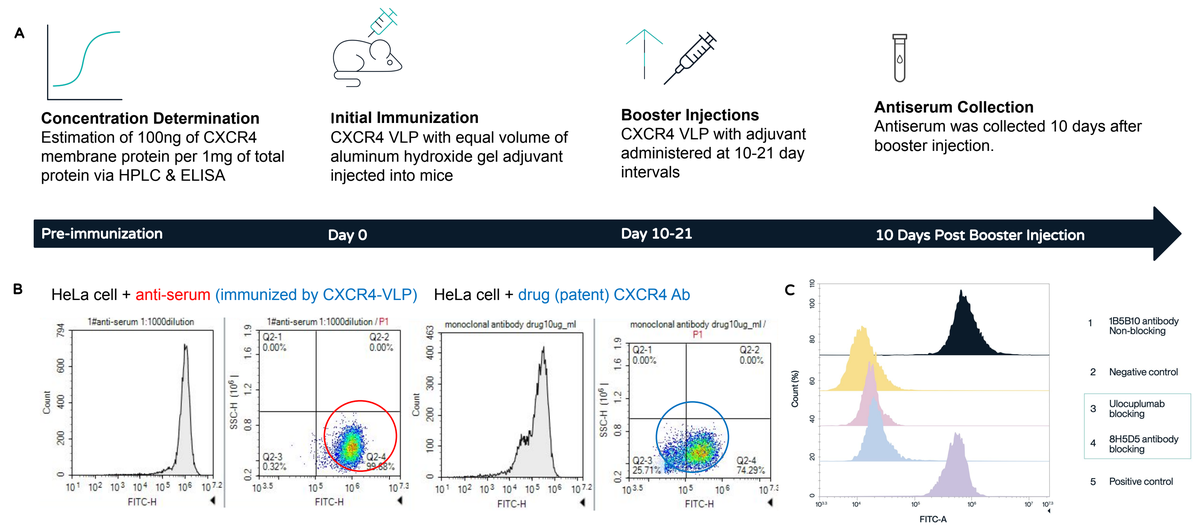

Figure 3. **(A)** Workflow of immune titer generation study of CXCR4 VLP. **(B)** CXCR4 antiserum titer evaluation via CXCR4-expressing HeLa cell sorting, using Ulocuplumab as a positive control. **(C)** Flow cytometry analysis of ligand blocking by commercial anti-CXCR4 monoclonal antibody as well as anti-CXCR4 monoclonal antibodies isolated in-house from immunization campaign. The positive control (Panel 5) shows successful ligand binding to CXCR4. The 8H5D5 antibody (Lane 4) and the commercial blocking antibody Ulocuplumab (Lane 3) effectively blocked ligand binding, demonstrating a shift in fluorescence intensity similar to each other. The 1B5B10 antibody (Panel 1), classified as non-blocking, shows no inhibition of ligand binding, comparable to the negative control (Lane 2). This confirms that the 8H5D5 monoclonal antibody has similar blocking capability to the commercial CXCR4 blocking antibody.

Biotinylated VLPs & Nanodiscs for phage panning

Phage panning usually requires biotinylated proteins for optimal binding and screening. To fulfill this need, we developed biotinylated VLPs and Nanodiscs with full-length proteins displayed on their surface without interfering with the protein conformation. We showed that both our biotinylated VLP and Nanodiscs produced robust ELISA signals, demonstrating their ability to support phage panning, a screening process requiring strong binding interactions for effective target enrichment.

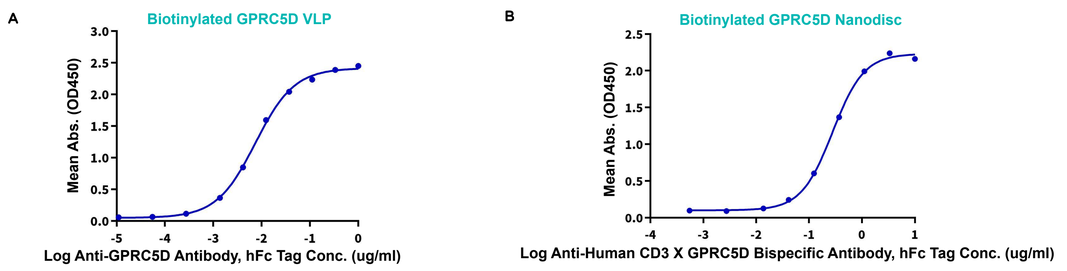

Figure 4. ELISA binding assays using biotinylated Human GPRC5D VLP and Nanodiscs. **Left:** Immobilized Biotinylated Human GPRC5D VLP at 5ug/ml (100ul/well) on the streptavidin precoated plate. Dose response curve for Anti-GPRC5D Antibody with the EC50 of 7.4 ng/ml. **Right:** Immobilized Human CD3E&CD3D at 2ug/ml (100ul/well) on the plate, add serial dilutions of Anti-Human CD3×GPRC5D Bispecific Antibody. Then add Biotinylated Human GPRC5D Nanodisc at 5ug/ml. Detection was performed using HRP-conjugated streptavidin with the EC50 of 0.28ug/ml.

Biologically-active TCR-CD3 complex Nanodisc for CD3 antibody screening

Traditionally, anti-CD3 antibodies are generated and screened using soluble CD3 subunits. These often show limited effectiveness as CD3 proteins naturally exist as part of the full TCR-CD3 complex on the T cell surface. Antibodies raised against individual subunits may fail to bind the native structure on the cell surface. To improve biological relevance and functionality, KACTUS has successfully produced nanodiscs containing the full complement of TCR-CD3 subunits, including 2XCD3ε, CD3γ, CD3δ, 2×CD3ζ, TCRα, and TCRβ, to facilitate antibody screening and lead isolation.

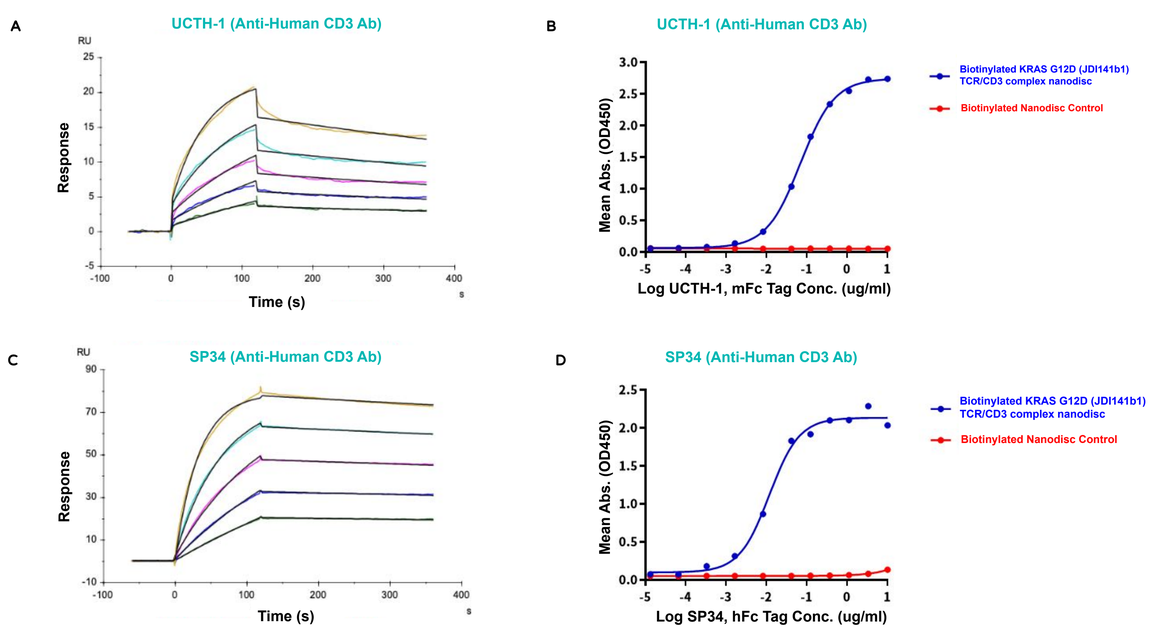

Figure 5. **(A)** SPR analysis of JDIa41b1 TCR-CD3 complex nanodisc against UCTH-1 antibody. Affinity Constant: 11.51 nM, **(B)** Immobilized TCR (JDIa41b1) TCR/CD3 complex Nanodisc at 2ug/ml (100 ul/well) on the streptavidin precoated plate. Dose Response curve for UCTH-1, mFc Tag with the EC50 of 69.2 ng/ml **(C)** SPR analysis of JDIa41b1 TCR-CD3 complex nanodisc against SP34 antibody. Affinity Constant: 0.1 nM. **(D)** Immobilized (JDIa41b1) TCR/CD3 complex Nanodisc, at 2ug/ml (100 ul/well) on the streptavidin precoated plate. Dose Response curve for SP34, hFc Tag with the EC50 of 11.8 ng/ml.

Conclusion

- KACTUS VLP and SMA-based nanodisc display platform allows robust, soluble display of membrane proteins with native structural conformation and robust bioactivity.
- KACTUS VLP and Nanodisc-displayed antigens are powerful tools for antibody discovery and bioanalytical assay development against membrane proteins, which can be applied for animal immunization, ELISA, SPR and BLI assays.

Membrane Proteins produced by KACTUS

We have successfully produced a variety of membrane proteins including GPCRs, Tetraspanins and GIPRs. To request a sample or learn more about our custom production capability, please contact us at support@kactusbio.us.