GPCR VLPs & Nanodiscs for Drug Discovery

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Introduction

G protein-coupled receptors (GPCRs), also known as seven-transmembrane receptors, represent the most important class of drug targets. However, extraction and purification of membrane proteins like GPCRs, ion channels, and transporters 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 VLP and/or nanodisc formats, which have been successfully applied to GPCR antibody drug discovery and various in vitro assays.

Full-length GPCR displaying 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 in antibody drug discovery and as a tool for studying antigens including GPCRs. VLPs can be engineered to display specific GPCRs on their surface, making them useful for stimulating an immune response against particular GPCRs.

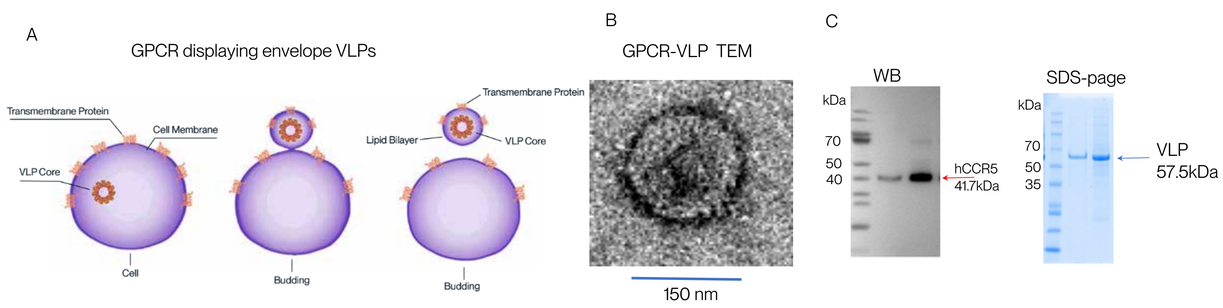
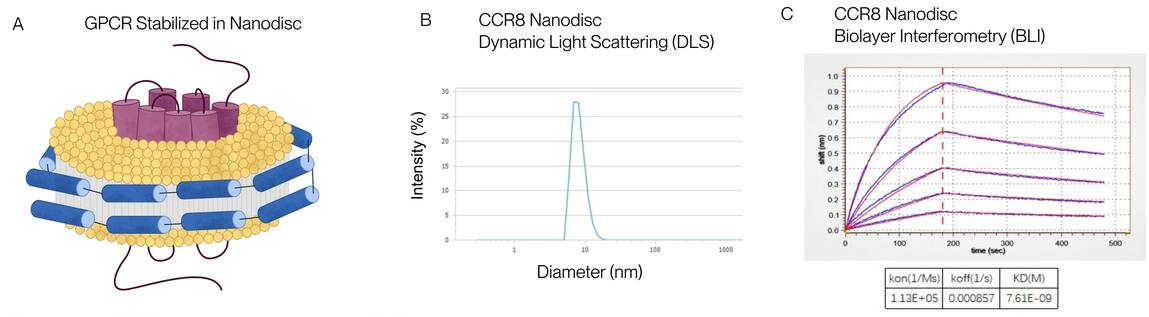


Figure 1. (A) By displaying the GPCRs on VLPs, the particles can trigger a robust immune response without the risk of causing disease, as the VLPs themselves are non-infectious. (B) TEM image indicates GPCR/VLP is around 150nm in diameter. (C). WB detects 41.7kDa hCCR5 membrane protein on VLP; 57.5kDa VLP protein band from hCCR5/VLP is observed on SDS-page.

Full-length GPCR assembled into nanodisc

Nanodiscs have emerged as a powerful tool in functional and structural studies of GPCR. Membrane scaffold protein, Salipros, copolymer nanodiscs , which are different approaches of membrane protein extraction and assembly, have been developed. For instance, copolymer nanodiscs can incorporate GPCRs in their endogenous lipids via extracting the GPCR directly from the cell membranes. The assembled GPCR nanodiscs are soluble in aqueous media in a native-like bilayer environment that maintains GPCR's activity.



kon(1/Ms)	koff(1/s)	KD(M)
1.13E+05	0.000857	7.61E-09

Figure 2. (A) GPCR assembled into nanodisc. GPCR transmembrane segments are stabilized by phospholipids and membrane scaffold proteins/polymers. GPCR intracellular and extracellular parts are exposed. (B) CCR8 is assembled in copolymer nanodisc, in 8.1nm diameter as measured by DLS . (C) Anti-CCR8 antibody immobilized on Protein A Chip can bind CCR8 nanodisc with an affinity constant of 7.6 nM as determined in BLI assay (Gator).

Case study 1: CXCR4-VLP immunization and antibody screening

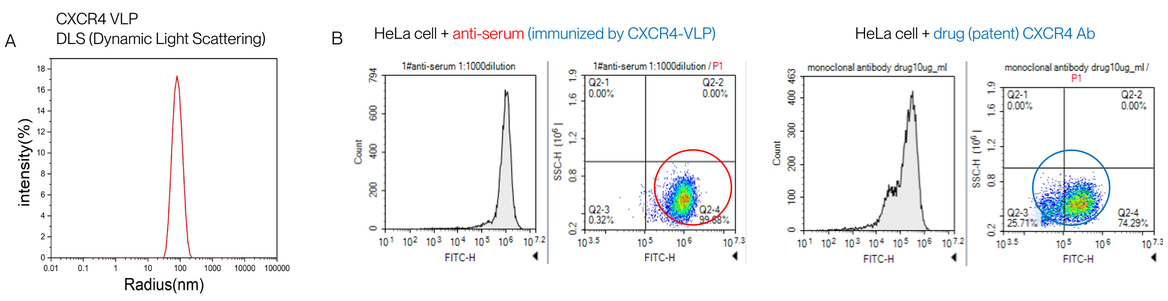
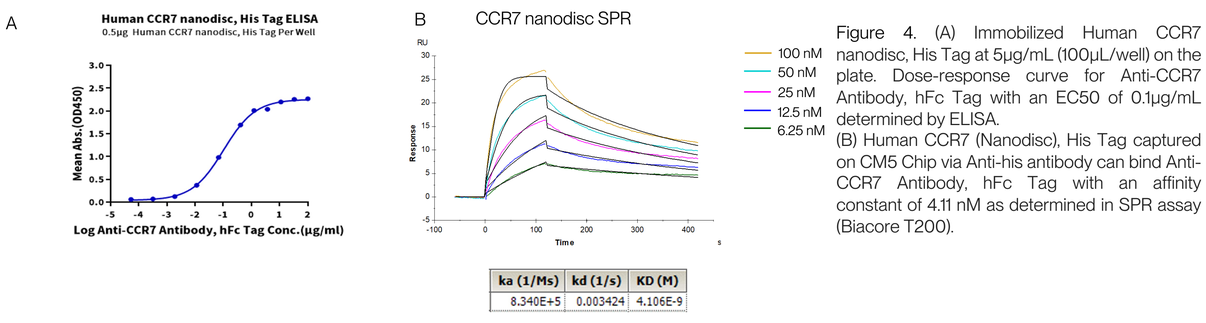


Figure 3. The whole process of GPCR/VLP immunization and hybridoma screening was explored, and high-affinity antibodies and SDF1 ligand-blocking antibodies were identified. (A) Dynamic Light Scattering (DLS) shows CXCR4/VLP in ~100nm radius. (B) Anti-serum binds with CXCR4 overexpression Hela cell line, showing positive FACS signal;Drug antibody binds to Hela cell line as FACS positive control. *Protocol for immunization:* CXCR4/VLP 0.5mg/ml total protein concentration was estimated by HPLC, 100ng CXCR4 membrane protein/1mg total protein was estimated by Elisa, binding with Ulocuplumab. 120 ul CXCR4/VLP combined with equal volume aluminum hydroxide gels (Alum) adjuvant for first immunization injection; Followed by 60ul CXCR4/VLP with adjuvant 4 time immune boosts at two week interval. (C) 8H5D5 and 1B5B10, high affinity antibodies against CXCR4 were obtaind. 8H5D5 antibody blocks SDF1 ligand binding to CXCR4, while 1B5B10 has no blocking effect.

Case Study 2: Human CCR7 nanodisc

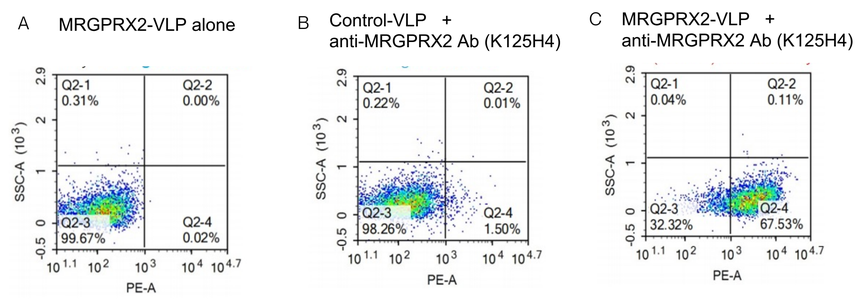
CCR7 is a promising target for immune therapy. CCR7 and its ligands CCL19 and CCL21 regulate homing of immune cells. CCR7 axis also plays a significant role in controlling the migration of tumor cells towards the lymphatic system and metastasis. Research indicates that the sensitivity of CCR7 to its ligand chemokines correlated with the levels of CCR7 homo- and CXCR4/CCR7 heterodimerization. KACTUS CCR7 membrane protein is expressed in CXCR4 knock-out HEK293 cell line and assembled as nanodisc. CCR7 nanodisc binds with drug antibody (Figure 3).



ka (1/Ms)	kd (1/s)	KD (M)
8.340E+5	0.003424	4.106E-9

Figure 4. (A) Immobilized Human CCR7 nanodisc, His Tag at 5μg/mL (100μL/well) on the plate. Dose-response curve for Anti-CCR7 Antibody, hFc Tag with an EC50 of 0.1μg/mL determined by ELISA.
(B) Human CCR7 (Nanodisc), His Tag captured on CM5 Chip via Anti-his antibody can bind Anti-CCR7 Antibody, hFc Tag with an affinity constant of 4.11 nM as determined in SPR assay (Biacore T200).

Case study 3: FACS Compatible MRGPRX2-VLP

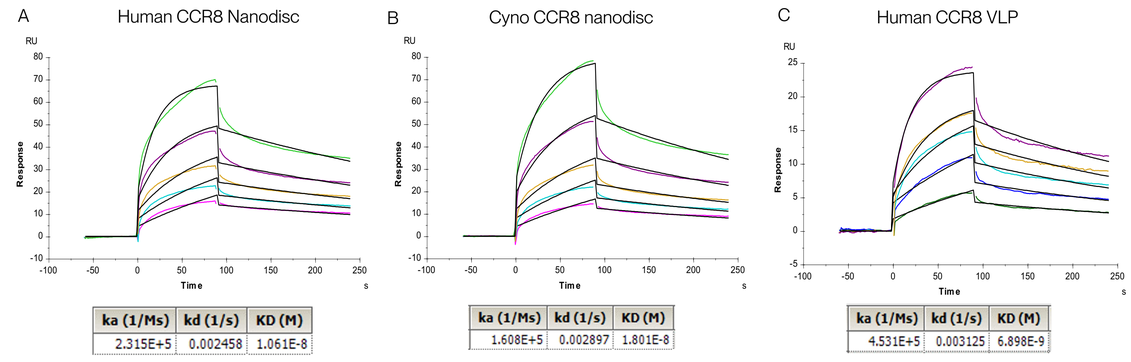


GPCR/VLP (~150nm in diameter) can be detected by FACS. Each VLP displays multiple GPCR molecules. Fluorescent-labeled GPCR-VLP is potentially useful in flow cytometry to identify GPCR-specific hybridoma.

Figure 5. (A) MRGPRX2-VLPs are detected by FACS.
(B) Control VLP has no binding with PE-labeled anti-MRGPRX2 antibody.
(C) MRGPRX2-VLPs bind with PE-labeled anti-MRGPRX2 antibody, fluorescent signal observed in PE channel of FACS.

Case study 4: CCR8-VLP for phage panning

SPR results of CCR8 nanodisc and CCR8/VLP binding with drug antibody are shown below. SPR is sensitive to low-affinity interactions like MHC/TCR (KD: 1*E-5M / 100μM). ELISA only works on high-affinity interactions. CCR8 nanodisc ELISA gives a very weak signal, which is consistent with the SPR result. CCR8/VLP can generate an ELISA signal strong enough for phage panning.



ka (1/Ms)	kd (1/s)	KD (M)
2.315E+5	0.002458	1.061E-8

ka (1/Ms)	kd (1/s)	KD (M)
1.608E+5	0.002897	1.801E-8

ka (1/Ms)	kd (1/s)	KD (M)
4.531E+5	0.003125	6.898E-9

Figure 6. SPR of CCR8 nanodisc and CCR8/VLP binding with drug antibody. The drug antibody is capable of cross-species binding with (A) human CCR8 nanodisc and (B) cynomolgus macaques CCR8 nanodisc . (C) KD indicates hCCR8/VLP has higher binding affinity with the drug antibody than hCCR8 nanodisc.

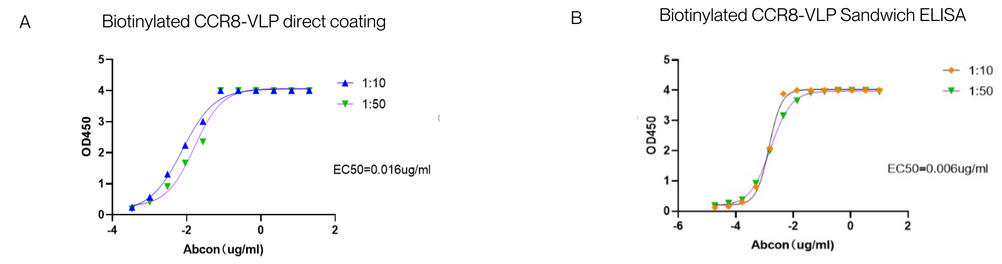


Figure 7. Biotinylated CCR8/VLPs were tested in ELISA. Indirect ELISA can be applied in antibody screening with efficient CCR8/VLP usage. *CCR8-VLP for phage panning protocol:* Dilute CCR8-VLP 1:50 in PBS. Coat with 100uL. Centrifuge at 3000rpm for 5 min. Store at 4°C overnight. The next day, wash with 1X PBS-T once. Then block with PBS-T with 3% BSA at 37 °C for 1hr. Then, it is ready for panning. 0.4mg CCR8/VLP , 0.4mg/ml, 1:50 dilution, 50ml CCR8-VLP is enough for 5*96 reactions. (A) Indirect ELISA: CCR8-VLP 1:10; 1:50 dilution, directly coated on ELISA plate, 100ul each well, with Anti-CCR8 drug antibody binding at 10ug/ml, 3x gradient dilution, Goat anti-human Fc-HRP for color development. (B) Sandwich ELISA: Streptavidin SA 20ug/ml, 100ul/well was coated onto ELISA plate. Biotinylated CCR8-VLP (1:10; 1:50 dilution) 100ul/well was captured. Anti-CCR8 drug antibody binding at 10ug/ml, 3x gradient dilution, Goat anti-human Fc-HRP for color development.

Case study 5: GPRC5D VLP & nanodisc for yeast display screening

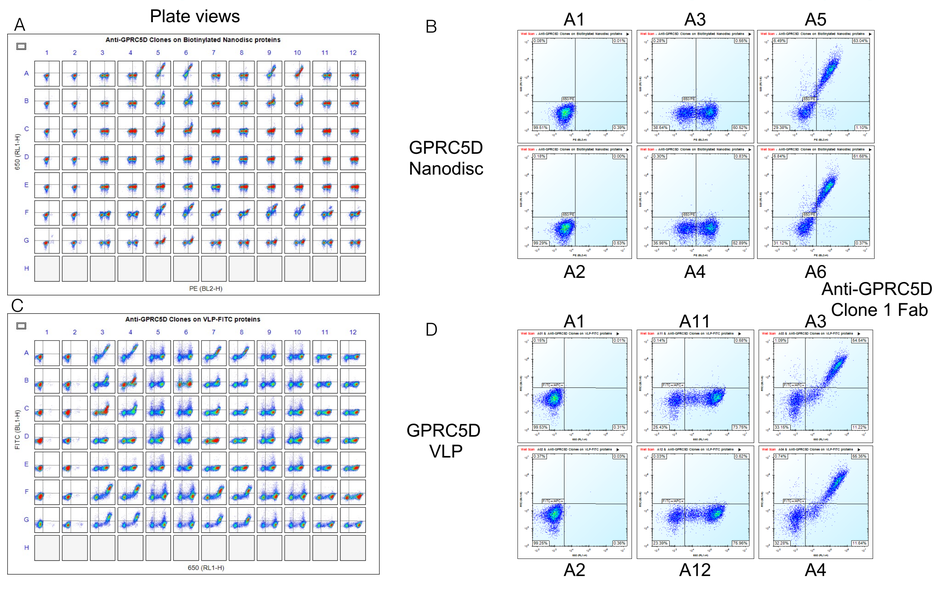


Figure 8. Nanodisc and VLP applications on Yeast Display screening (AvantGen, Inc.)
(A) FACS plate view on screening
(B) Panel A1/A2 two yeast display clones, no staining; Panel A3/A4 Yeast display clones, secondary antibody detection only, indicating antibody displayed. Panel A5/A6 Yeast display clones staining with biotinylated GPRC5D nanodisc (Copolymer) +SA-APC
(C) FACS plate view on screening.
(D) Panel A1/A2 two yeast display clones, no staining; Panel A11/A12 yeast display clones, staining with FITC equivalent GPRC5D/VLP. Panel A3/A4 yeast display clones, bind APC-anti-IgG, indicating antibodies exist.

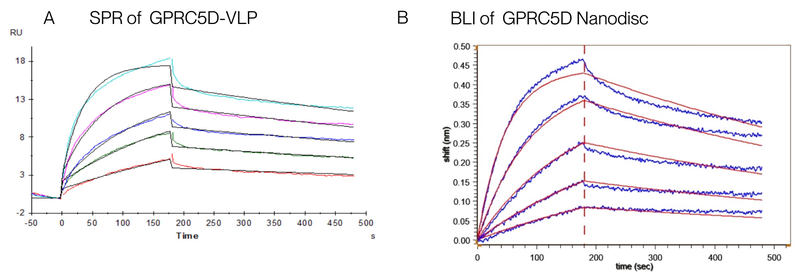


Figure 9. Biotinylated version of GPCR VLP and nanodisc can be used in ELISA, SPR, and BLI.
(A) Biotinylated Human GPRC5D VLP captured on SA Chip can bind anti-GPRC5D antibody, hFc with an affinity constant of 0.30 nM as determined in SPR assay (Biacore T200).
(B) When the His tag is fused at the intracellular terminus of GPCR, the nanodisc can be captured by anti-His tag antibody. Human GPRC5D Nanodisc, His Tag immobilized on His-biosensor, can bind anti-GPRC5D antibody with an affinity constant of 0.58 nM as determined in BLI assay (Gator).

Conclusion

1. KACTUS proprietary membrane proteins cover series of GPCRs, ion channels, transporters etc. in VLP and/or nanodisc formats.
2. Full-length GPCRs or engineered GPCR domains possessing critical post-translational modifications are displayed on VLP, which are well suited for immunization and antibody discovery. CXCR4/VLP , CCR8/VLP serve as case studies.
3. GPCR/VLP itself can be detected by FACS, multiple GPCR molecules are displayed on VLP, making it potentially useful in flow cytometry to identify GPCR-specifc hybridoma.
4. Intracellular domains of GPCR are exposed in nanodisc format, making it applicable for certain in vitro assays. Moreover, there are cases that GPCR nanodisc is obtainable while GPCR/VLP is difficult, CCR7 nanodisc serves as an example.
5. GPCR/VLP and GPCR nanodisc (copolymer) can be used for yeast display screening and in SPR, BLI for binding kinetics.

References

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