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.

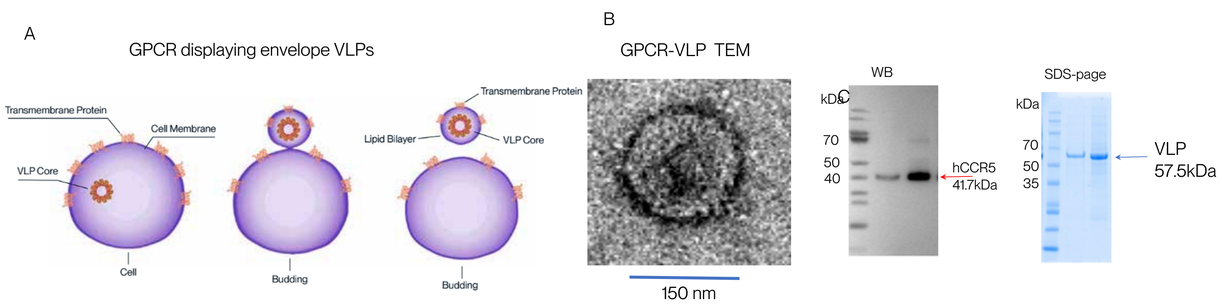


Figure 1. (A) By displaying the GPCRs on VLPs, the particles can trigger a robust immune response without the risk of causing disease, as the VLPs themselves are non-infectious. (B) TEM image indicates GPCR/VLP is around 150nm in diameter. (C). WB detects 41.7kDa hCCR5 membrane protein on VLP; 57.5kDa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

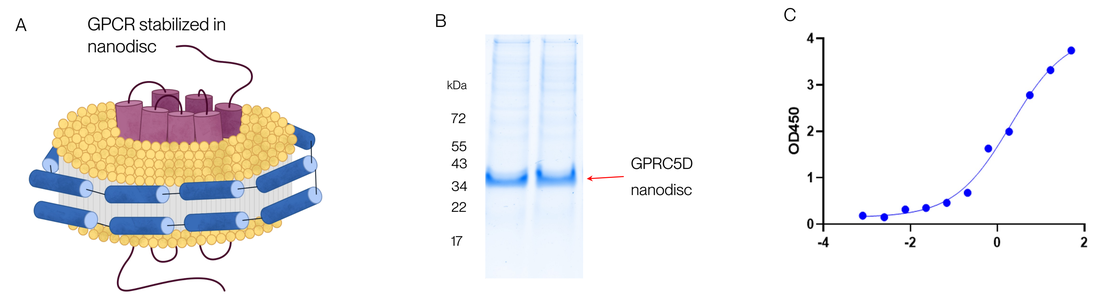


Figure 2. (A) GPCR assembled into nanodisc. GPCR transmembrane segments are stabilized by phospholipids and membrane scaffold proteins /polymers. GPCR intracellular and extracellular parts are exposed. (B) GPRC5D is assembled in copolymer nanodisc, GPRC5D protein band is visible on SDS-page. (C) To immobilize nanodisc onto ELISA plate, GPRC5D-His tag nanodisc was captured by anti-His tag antibody, which was precoated on ELISA plate. Biotinylated avi tag on GPCR can serve the same purpose. GPCR nanodisc can be used for drug screening.

Case study 1: CXCR4-VLP immunization and antibody screening

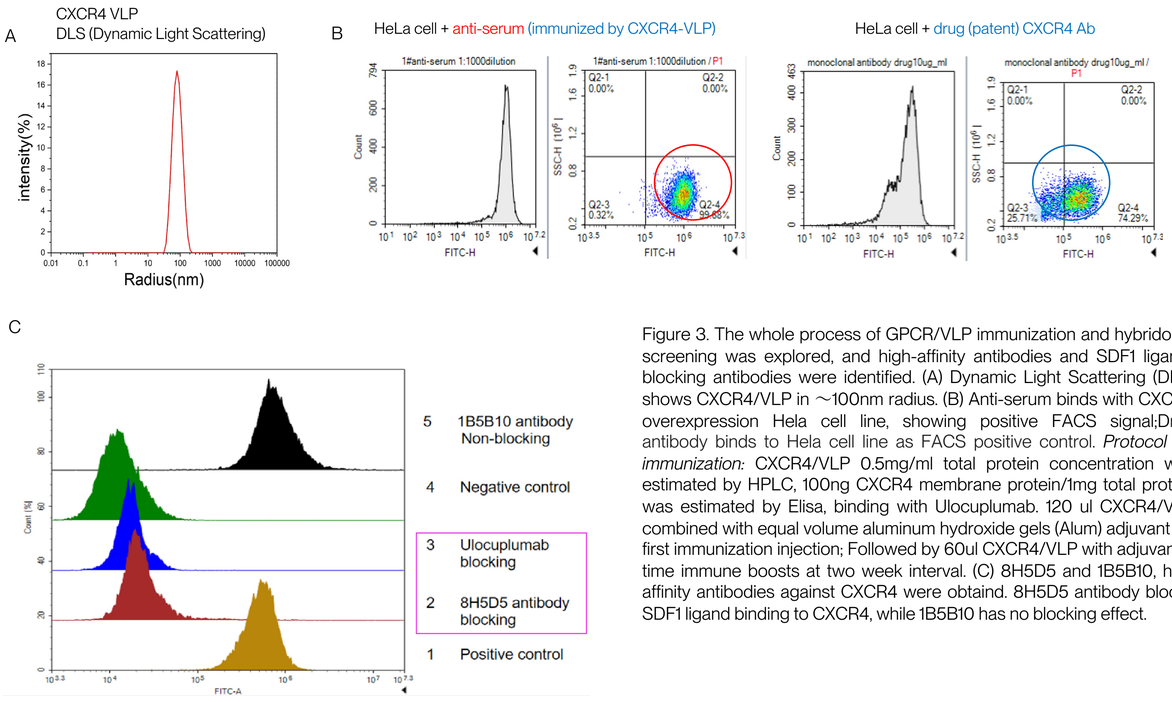
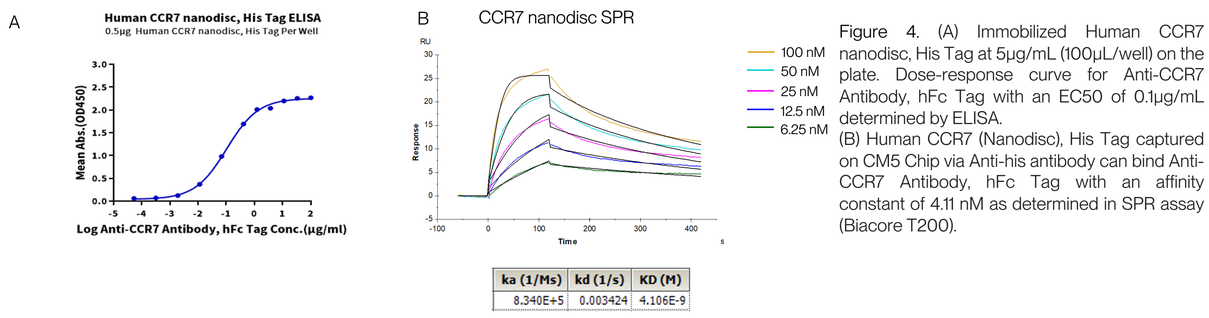


Figure 3. The whole process of GPCR/VLP immunization and hybridoma screening was explored, and high-affinity antibodies and SDF1 ligand-blocking antibodies were identified. (A) Dynamic Light Scattering (DLS) shows CXCR4/VLP in ~100nm radius. (B) Anti-serum binds with CXCR4 overexpression Hela cell line, 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 GPCR-VLPs

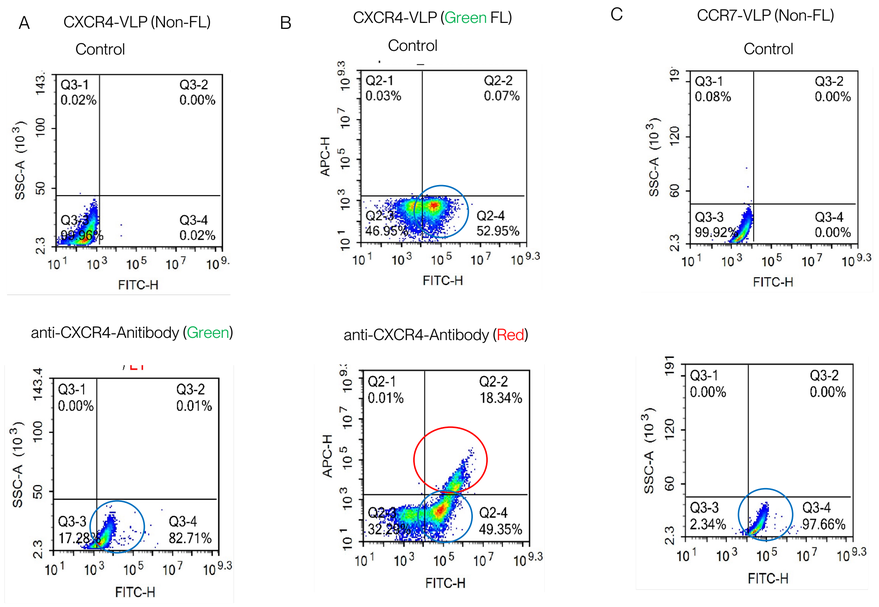
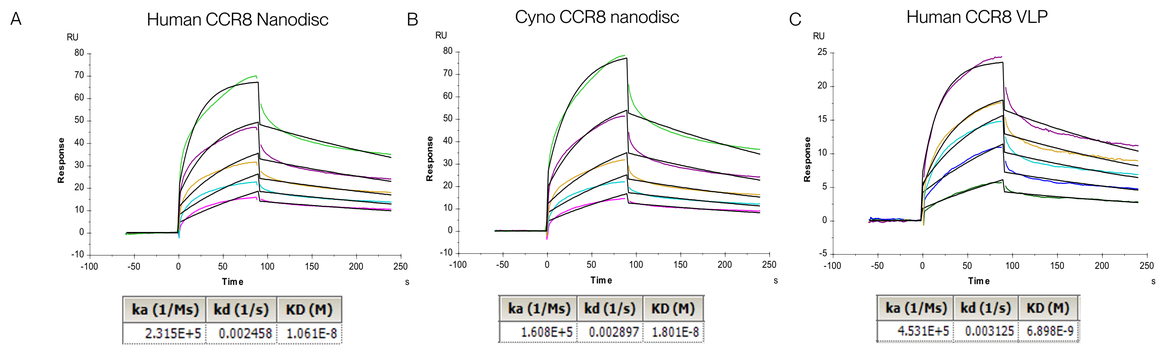


Figure 5. GPCR/VLP (~150nm in diameter) can be detected by FACS. Multiple GPCR molecules are displayed on each VLP. Fluorescent-labeled GPCR-VLP is potentially useful in flow cytometry to identify GPCR-specific hybridoma. (A, C, upper panel) CXCR4/VLP, CCR7/VLP show no signal in FACS FITC channel. (A, C lower panel) FITC labeled anti-CXCR4 antibody and FITC labeled anti-CCR7 antibody binds with CXCR4/VLP and CCR7/VLP respectively and gives signal in FACS FITC channel. (B, upper panel) Fluorescent-labeled CXCR4/VLP shows signal in FACS FITC channel. (B, lower panel) Fluorescent-labeled CXCR4/VLP binds with APC labeled anti-CXCR4 antibody and gives signal in FACS APC channel.

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-5 M/ 100uM). 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.

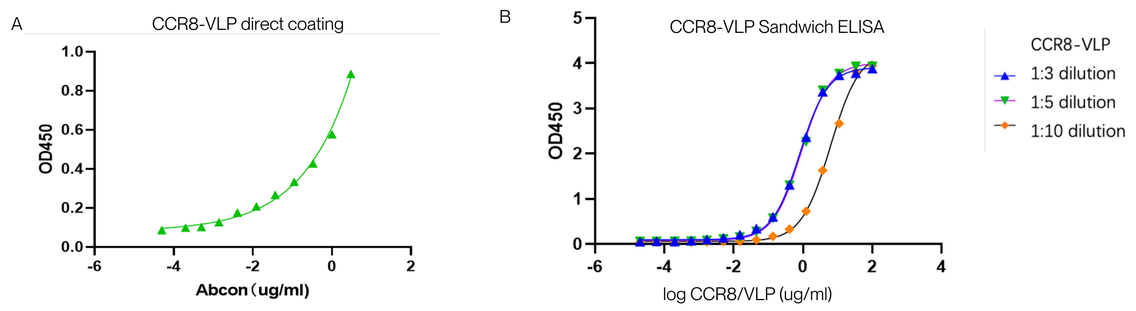
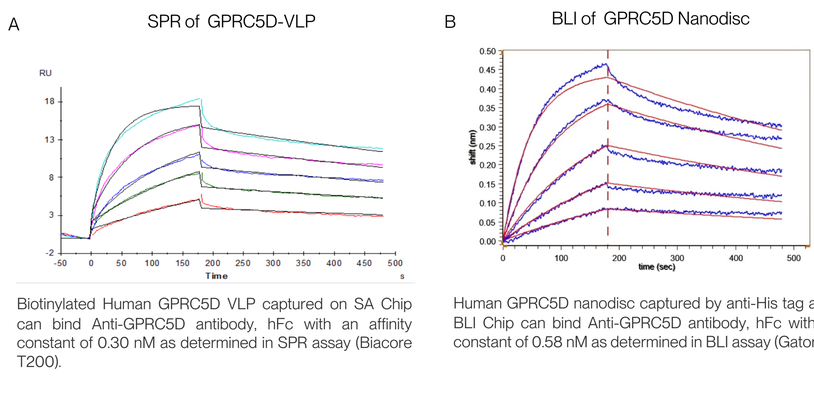


Figure 7. CCR8 nanodisc and CCR8/VLP were tested in ELISA. Indirect ELISA can be applied in antibody screening with efficient CCR8/VLP usage. *CCR8-VLP for phage panning protocol:* Dilute CCR8-VLP 1:5 in PBS. Coat with 100uL. Centrifuge at 3000rpm for 5 min. Store at 4°C overnight. The next day, wash with 1X PBS-T once. Then block with PBS-T with 3% BSA at 37 °C for 1hr. Then, it is ready for panning. 1mg CCR8/VLP , 0.3mg/ml, 1:5 dilution, 10ml CCR8-VLP is enough for 96 reactions. (A) Indirect ELISA: CCR8-VLP estimated 0.3mg total protein/ml, 1:4 dilution, directly coated on ELISA plate, 100ul each well, with biotinylated drug antibody at 3ug/ml, 3x gradient dilution for detection. (B) Sandwich ELISA: Drug antibody 20ug/ml, 100ul/well was coated onto ELISA plate. CCR8-VLP (1:3; 1:5; 1:10 dilution) 100ul/well was captured. The biotinylated drug antibody (3ug/ml) was used for detection at 3X gradient dilution.

Case study 5: GPCR-VLP & GPCR nanodisc binding kinetics



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).

Human GPRC5D nanodisc captured by anti-His tag antibody on BLI Chip can bind Anti-GPRC5D antibody, hFc with an affinity constant of 0.58 nM as determined in BLI assay (Gator).

Figure 8. Biotinylated version of GPCR 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. Loaded Biotinylated Human GPRC5D Nanodisc, His Tag on SA-Biosensor, can bind Anti-GPRC5D Antibody with an affinity constant of 1.16 nM as determined in BLI assay (Gator Prime).

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 can be used in SPR, BLI for binding kinetics.

References

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