Recombinant Bioactive CXCR4 or CXCR5 on Virus-Like Nanoparticles Can Bind to Their Chemokine Ligand CXCL12 or CXCL13

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Abstract

CXCR4 and CXCR5 are G-protein coupled receptors (GPCRs). CXCR4 is a receptor for chemokine CXCL12 and a co-receptor for HIV to infect CD4 T-cells. CXCR5 is a receptor for chemokine CXCL13. The overexpression of CXCR4 and CXCR5 on cancer cells makes them attractive targets for therapeutics. As seven transmembrane GPCRs, it is very challenging to generate bioactive chemokine receptor recombinant proteins with a native conformation. We engineered virus-like particles (VLPs) to display CXCR4 or CXCR5 with enhanced surface expression and embedded in the lipid bilayer as nanoparticles, CXCR4-VLP or CXCR5-VLP, respectively. The CXCR4-VLP and CXCR5-VLP nanoparticles were expressed using HEK293T cells. The purified nanoparticles were tested for size and morphology using electron-microscopy and dynamic light scattering. The target proteins were analyzed by multiple tests including antibody and ligand binding assays. Both CXCR4-VLP and CXCR5-VLP could bind potently to its specific antibody with EC50 values <100 ng/ml. More importantly, they could interact with their ligands as measured by ELISA. CXCR4-VLP could bind to its chemokine ligand CXCL12 and to HIV-1 envelope glycoprotein gp120. CXCR5-VLP could bind to its chemokine ligand CXCL13. The biotinylated and non-biotinylated CXCR4-VLP could bind to the specific antibody very similarly. The results imply that these novel CXCR4-VLP and CXCR5-VLP nanoparticles are bioactive, can potentially be used to evaluate the chemokine/receptor interactions, and can be used as antigens for drug discovery.

CXCR4-VLP (Conigen Bioscience, VLP-24005); CXCR5-VLP (Conigen Bioscience, VLP-24014)

Results

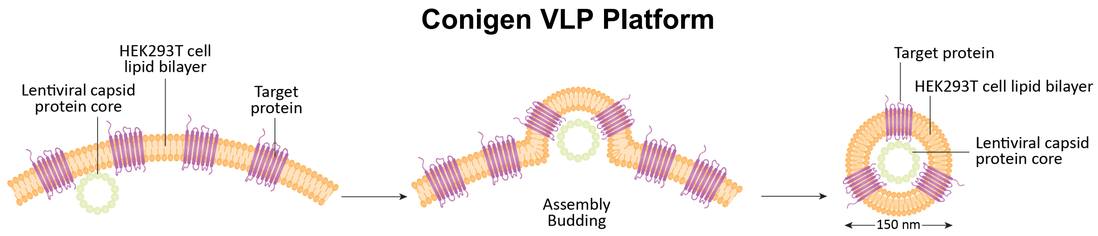

Fig. 1. Conigen Membrane Protein (CMP) platform enhances the target density and nanoparticle production yield by optimizing the expression of the GPCR target on VLP nanoparticles in HEK293T cells.

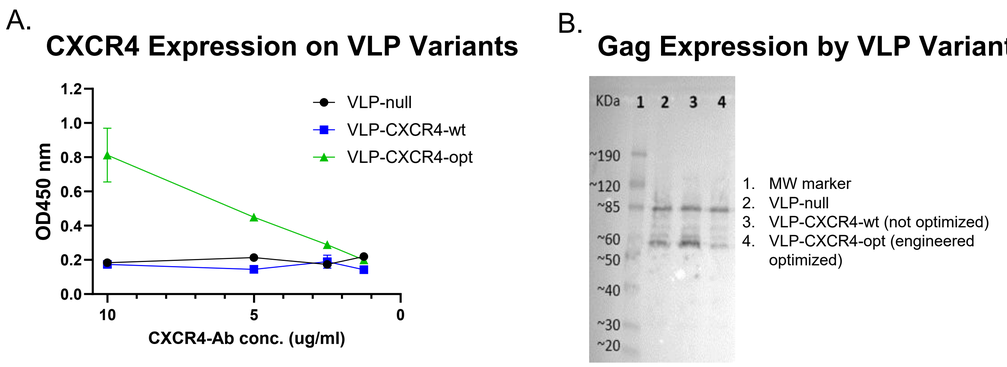

Fig. 2. Optimization of CXCR4 target surface expression on VLP. (A) Detection of CXCR4 expression on VLP by ELISA. (B) Detection of Gag-expression in VLP by Western blot.

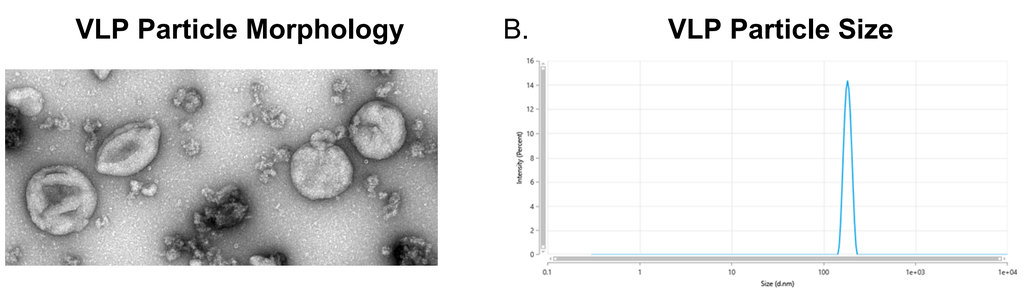

Fig. 3. Examples of VLP morphology by electron microscope (negative straining (A) and size measurement as measured by dynamic light scattering (B) The nanoparticle sizes are 150-200 nm in diameter.

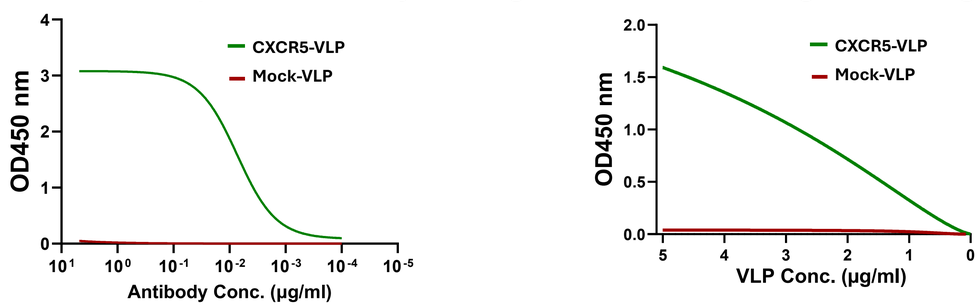

Fig. 4. CXCR5-VLP binding to the specific antibody (A) and CXCL13 chemokine ligand (B)..

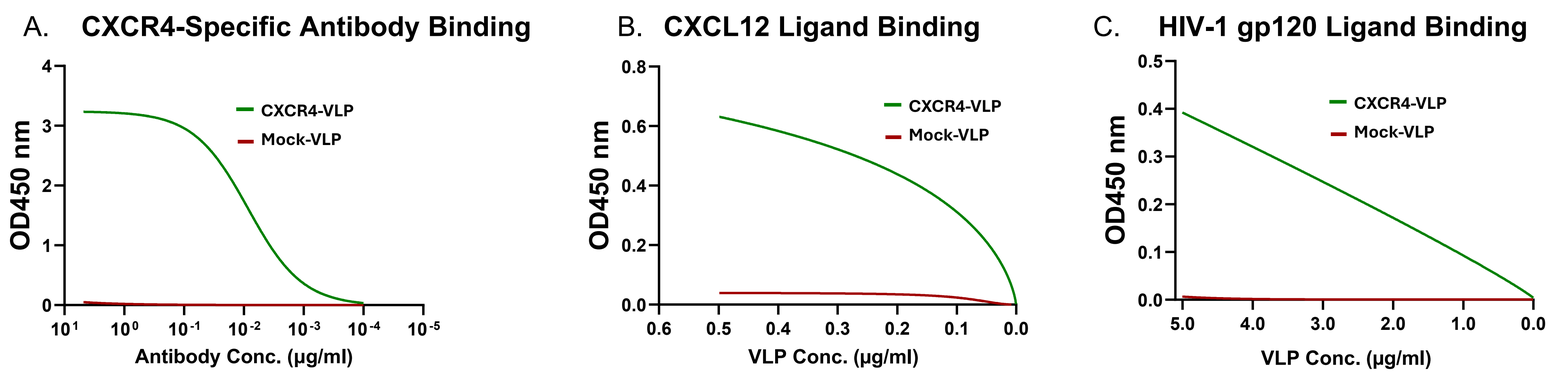

Fig. 5. CXCR4-VLP binding to the specific antibody (A), CXCL12 chemokine ligand (B) and HIV-1 gp120 ligand (C).

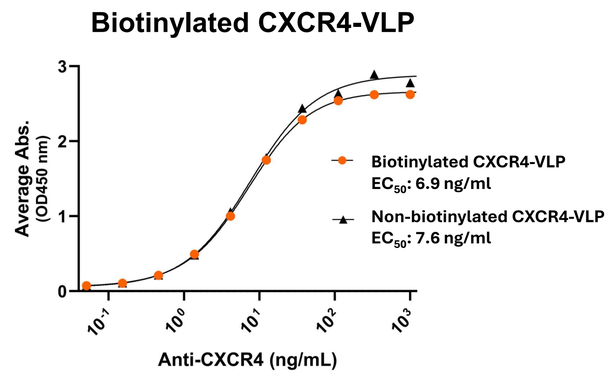

Fig. 6. Biotinylated CXCR4-VLP binding to specific antibody similarly to the non-biotinylated CXCR4-VLP.

Conclusions

- Optimization of GPCR target surface expression and particle release enhance the GPCR expression on VLP nanoparticles and the productivity.
- CXCR4 and CXCR5 expressed on the surface of VLP nanoparticles can potently bind to the specific antibodies.
- CXCR4 and CXCR5 target proteins on the VLP nanoparticles present the correct native conformation and are bioactive in binding their ligands.
- The VLP biotinylation preserves the integrity of the GPCR target protein.
- The bioactive CXCR4-VLP and CXCR5-VLP nanoparticles can serve as antigens and immunogens for research and therapeutic discovery

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