Recombinant G-Protein Coupled Chemokine Receptors CXCR4 and CXCR5 on Nanoparticles are Bioactive, Binding to the Chemokine Ligands

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Abstract

The selective chemokine and receptor interactions are critical in inflammatory responses. CXCR4 and CXCR5 are G-protein coupled cytokine 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 make them attractive targets for therapeutics. As GPCRs are seven transmembrane receptors, 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-CMP or CXCR5-CMP, respectively. The CXCR4-CMP or CXCR5-CMP 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-CMP and CXCR5-CMP 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-CMP could bind to its chemokine ligand CXCL12 and to HIV-1 envelope glycoprotein gp120. CXCR5-CMP could bind to its chemokine ligand CXCL13. The results imply that these novel CXCR4-CMP and CXCR5-CMP nanoparticles are bioactive, can potentially be used to evaluate the chemokine/receptor interactions and can be used as antigens for drug discovery.

Materials and Methods

Recombinant Proteins: CXCR4-CMP and CXCR5-CMP nanoparticle proteins are expressed in HEK293T cells and purified using size-exclusion chromatography. CXCR4-CMP (Conigen Bioscience, CMP-24005, UniProt# P61073), CXCR5-CMP (Conigen Bioscience, CMP-24014, UniProt# P32302), CXCL12 cytokine (Acro Biosystems), CXCL13 cytokine (Acro Biosystems), HIV-1 gp120-JRFL protein (Worcester HIV Vaccine).
Antibody binding assays: CXCR4- or CXCR5-specific antibody binding was measured by ELISA. CXCR4-CMP or CXCR5-CMP protein was coated on 96-well microtiter plates at 2 μg/ml and detected by CXCR4- or CXCR5-specific antibodies at a series of concentrations.
Ligand/Receptor binding assays: ELISAs were performed to evaluate the ability of CXCR4 or CXCR5 to bind to their respective ligands: CXCL12 and HIV-1 gp120, or CLXCL13. The ligand protein was coated on 96-well microtiter plates at 5 μg/ml and detected by CXCR4-CMP or CXCR5-CMP at a series of concentrations.

Results

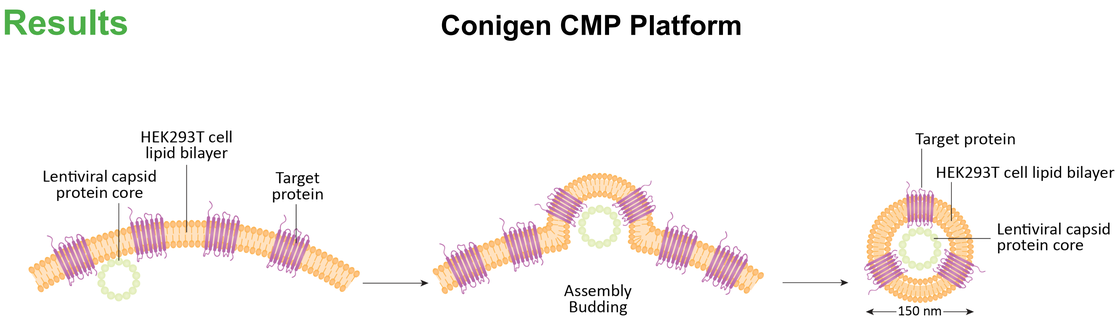

Fig. 1. Conigen Membrane Protein (CMP) platform enhances the target density and nanoparticle production yield by optimizing the expression of the GPCR target on VLP nanoparticles in HEK293T cells.

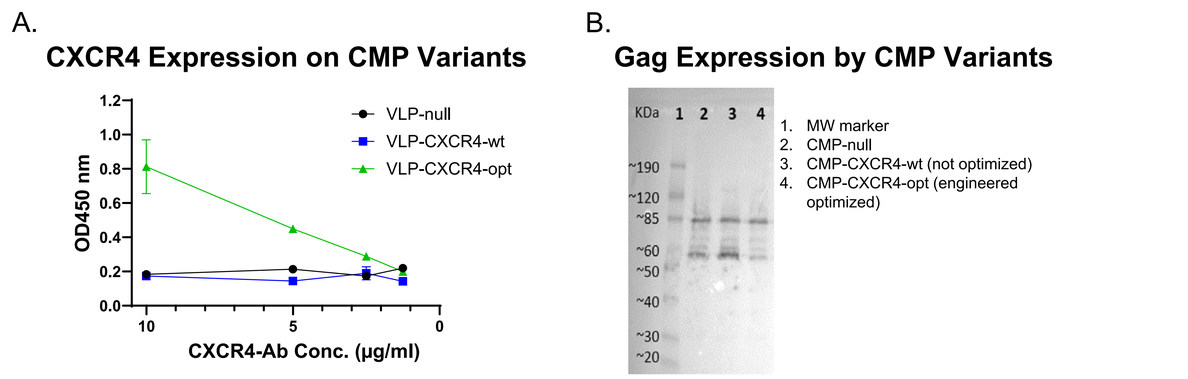

Fig. 2. Optimization of CXCR4 target surface expression. (A) Detection of CXCR4 expression by ELISA. (B) Detection of Gag-expression by Western blot.

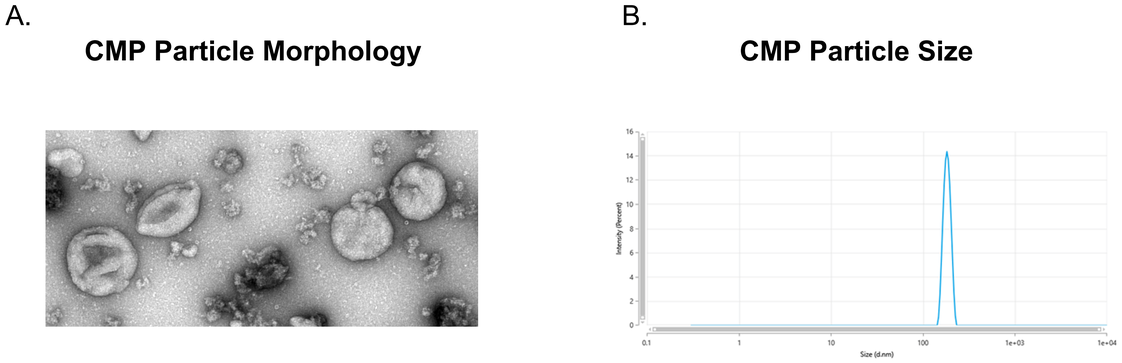

Fig. 3. Examples of CMP morphology by electron microscope (negative straining (A) and size measurement as measured by dynamic light scattering (B) The nanoparticle sizes are 150-200 nm in diameter.

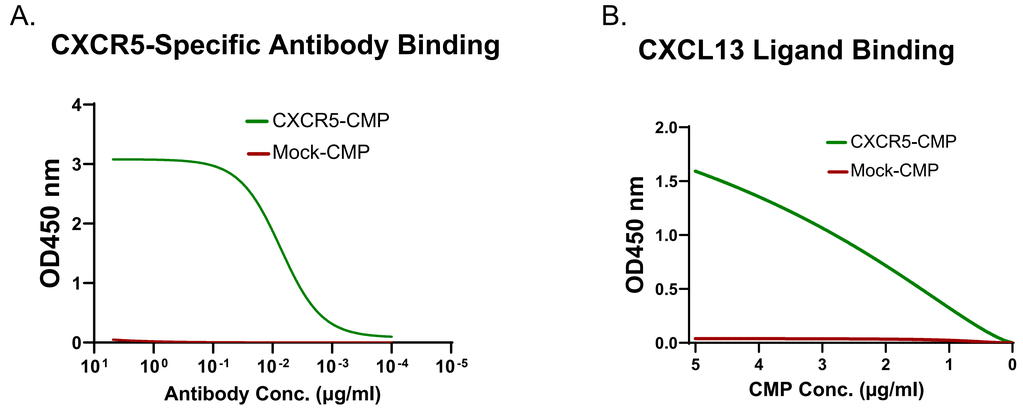

Fig. 4. CXCR5-CMP binding to the: (A) specific antibody and (B) CXCL13 chemokine ligand.

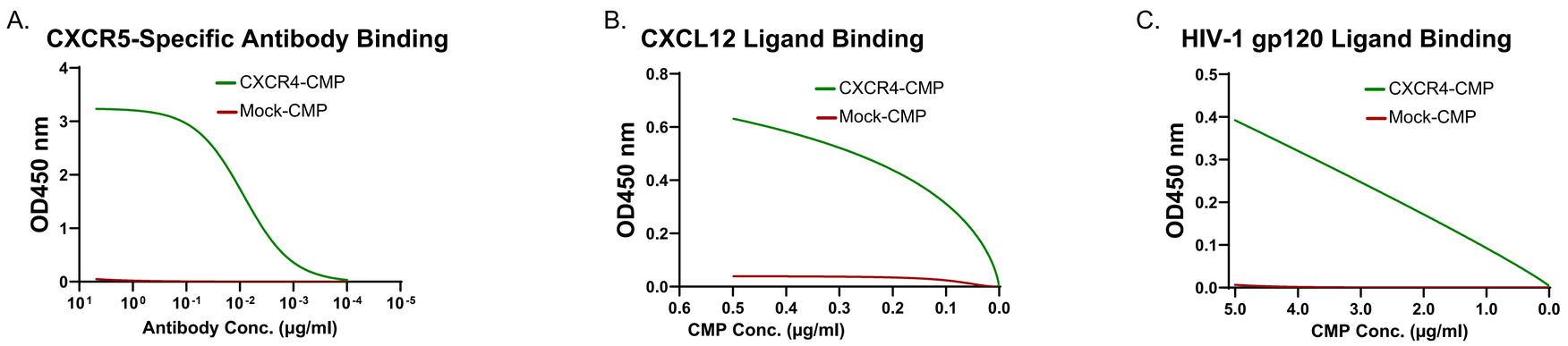

Fig. 5. CXCR4-CMP binding to the: (A) specific antibody, (B) CXCL12 chemokine ligand and (C) HIV-1 gp120 ligand.

Conclusions

- Optimization of the GPCR chemokine receptor surface expression and particle release enhance the GPCR-presentation on VLP nanoparticles and the productivity.
- CXCR4 and CXCR5 target proteins expressed on the VLP nanoparticles surface can potently bind to the specific antibodies.
- CXCR4 and CXCR5 target proteins on the VLP nanoparticles present the correct native conformation and are bioactive. CXCR5 can bind its chemokine ligand CXCL13; CXCR4 can bind its chemokine ligand CXCL12 and HIV-1 gp120 protein as a co-receptor.
- These bioactive CXCR4-CMP and CXCR5-CMP nanoparticles can serve as antigens and immunogens for research and therapeutic discovery

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