Multi-Pass Proteins Displayed on Virus-Like Particles Are Bioactive in Binding to Specific Antibodies and Ligands

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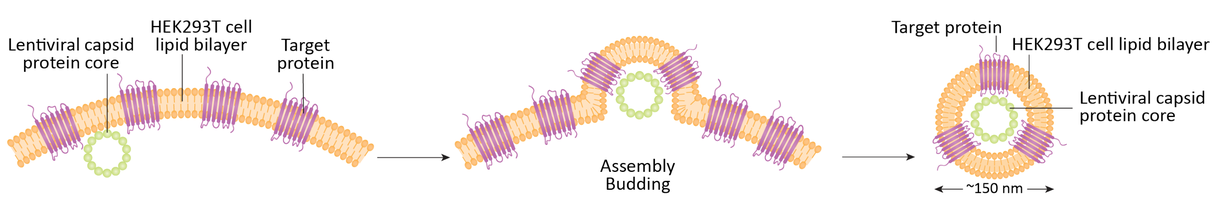
Abstract

Multi-pass proteins are integral membrane proteins containing two or more helical transmembrane domains (TMDs) that traverse the cell membrane lipid bilayer. These proteins, including G protein-coupled receptors (GPCRs), transporters, ion-channels and Claudin family proteins, regulate nearly every aspect of human physiology and diseases. They are one of the largest drug target families. GPCRs have 7-TMDs, glucose transporters have 12-TMDs and Claudins have 4-TMDs. Given the structure complexity, it is very difficult to produce recombinant proteins for research and drug discovery. We have developed an advanced platform to display multi-pass proteins with 4-12 TMDs in the natural membrane lipid bilayer on virus-like particles (VLPs) as antigen for antibody screening/characterization and receptor/ligand interaction analysis. We developed a platform to enhance multi-pass membrane protein expression on the VLP surface and properly embed it in the natural lipid bilayer of VLP nanoparticles. The target is co-expressed with a lentiviral structure protein using HEK293 cells. The purified VLP-nanoparticles are tested for size using dynamic light scattering. The target proteins were analyzed by multiple tests including antibody and ligand binding assays. In this project, we use two 4-TMD Claudin family proteins (Claudin 18.2 and Claudin 6), three 7-TMD GPCR chemokine receptors (CXCR4, CXCR5 and CX3CR1), and one 12-TMD glucose transporter GLUT1 as examples to generate target-expressing VLPs. The Claudin 18.2, Claudin 6, CXCR4, CXCR5 and GLUT1 VLPs could each bind potently to its specific antibody. The chemokine receptor VLPs could interact with their ligands as measured by ELISA. CXCR4-VLP could bind to its chemokine ligand CXCL12 and HIV-1 envelope glycoprotein gp120. CXCR5-VLP could bind to its chemokine ligand CXCL13. Conclusions: The multi-pass membrane proteins can be properly displayed on VLPs. These Claudin family VLPs, GPCR-VLPs, transporter and ion-channel VLPs can be used to evaluate receptor/ligand interactions and as antigen or immunogen for drug discovery.

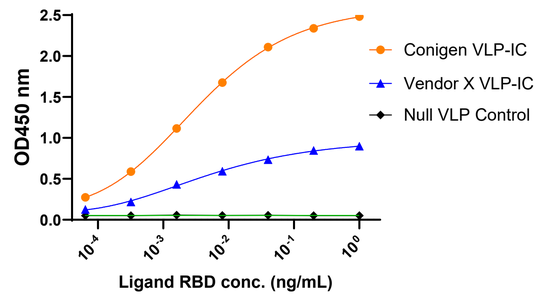
1. CMP™ Technology Platform: Display multi-pass membrane proteins on virus-like particles (VLPs)



Optimized to enhance the target density and nanoparticle production yield

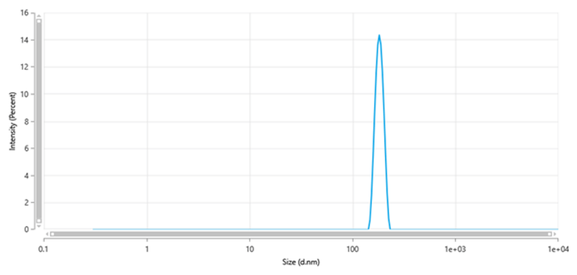
2. Examples of Conigen VLPs with higher densities (A) and nanoparticle sizes (B)

A. Conigen VLPs have much stronger ligand binding than other vendor VLPs



Ion channel target expressed on the surface of VLP produced by Conigen had much stronger ligand binding than from Vendor X, indicating higher target densities.

B. Conigen VLP nanoparticle sizes

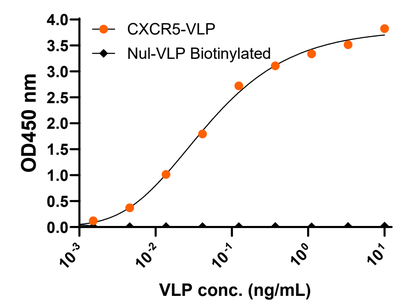


Conigen's VLP sizes are ~150nm in diameter, as measured by dynamic light scattering.

3. GPCR (7-TMD) chemokine receptors displayed on VLP can potently bind specific antibodies and ligands

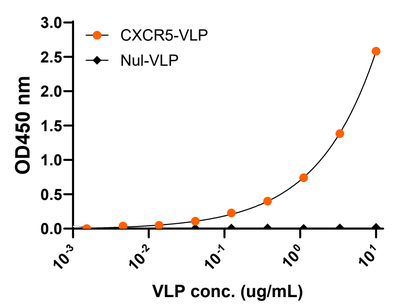
CXCR5-VLP binds specific antibody (A), CXCL12 chemokine ligand (B)

A. CXCR5-specific antibody binding



The CXCR5 target on the VLP can bind the CXCR5-specific antibody specifically and potently, EC50 < 0.02 ug/ml, as measured by ELISA.

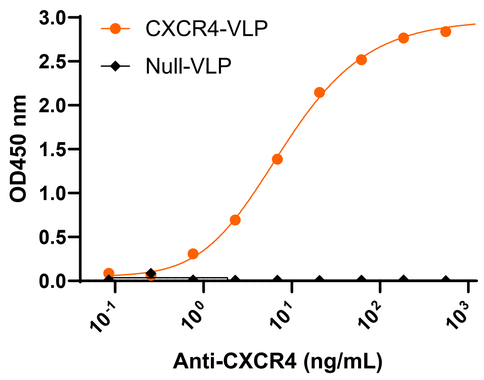
B. CXCL13 ligand binding



The CXCR5 target on the VLP can bind the ligand CXCL13, EC50 < 5 ug/ml, as measured by ELISA.

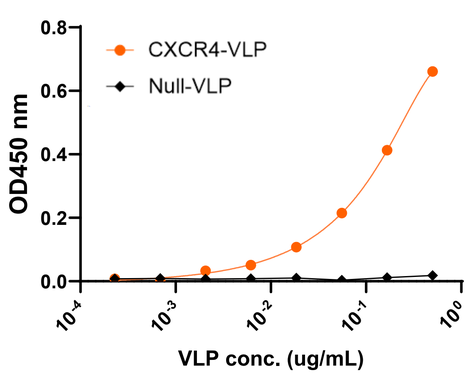
CXCR4-VLP binds specific antibody (C), CXCL12 chemokine ligand (D)

C. CXCR4-specific antibody binding



The CXCR4 target on the VLP can bind the CXCR4-specific antibody specifically and potently, EC50 < 0.02 ug/ml, as measured by ELISA.

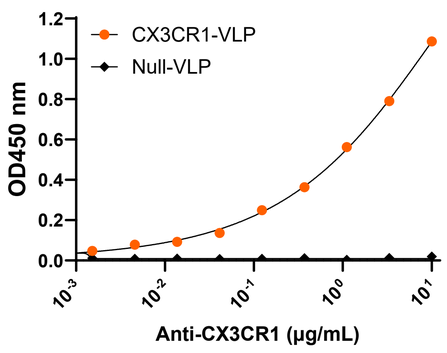
D. CXCL12 ligand binding



The CXCR4 target on the VLP can bind the ligand CXCL12, EC50 < 5 ug/ml, as measured by ELISA.

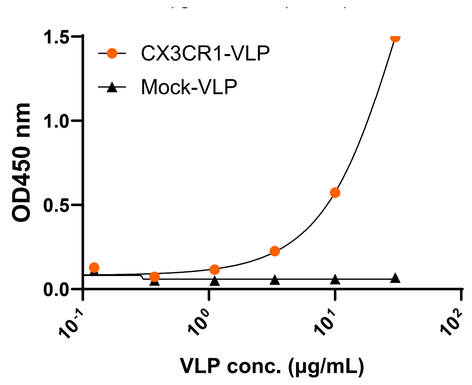
CX3CR1-VLP binds specific antibody (E), CX3CL1 chemokine ligand (F)

E. CX3CR1-specific antibody binding



The CX3CR1 target on the VLP can bind the CX3CR1-specific antibody specifically and potently, EC50 < 10 ug/ml, as measured by ELISA.

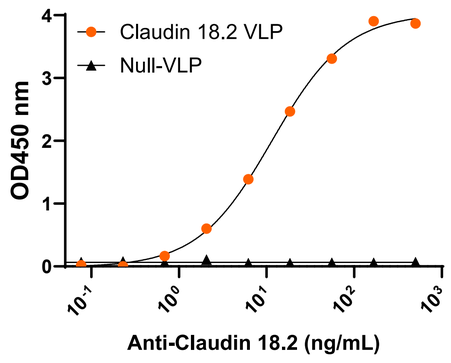
F. CX3CL1 ligand binding



The CX3CR1 target on the VLP can bind the ligand CX3CL1 EC50 < 15 ug/ml, as measured by ELISA.

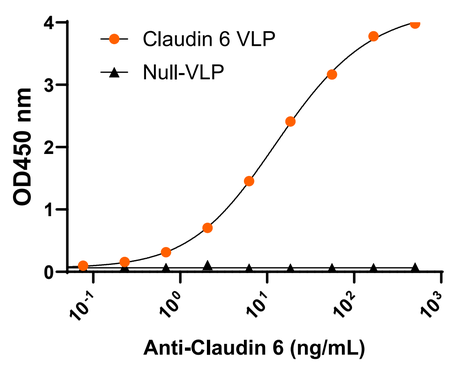
4. Claudin family (4-TMD) Claudin 18.2 (A) and Claudin 6 (B) VLPs

Claudin 18.2-specific antibody binding



The Claudin 18.2 VLP can bind the target specific antibody specifically and potently, EC50 < 0.01 ug/ml, as measured by ELISA.

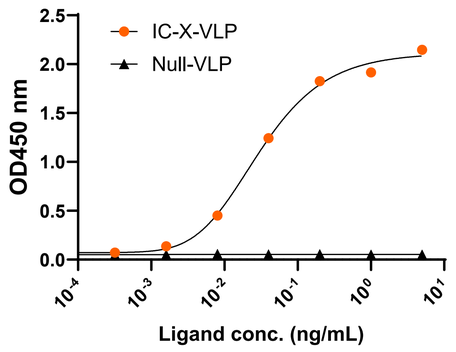
Claudin 6-specific antibody binding



The Claudin 6 VLP can bind the target-specific antibody specifically and potently, EC50 < 0.01 ug/ml, as measured by ELISA.

5. Ion channel IC-X (10-TMD) VLP binding to ligand

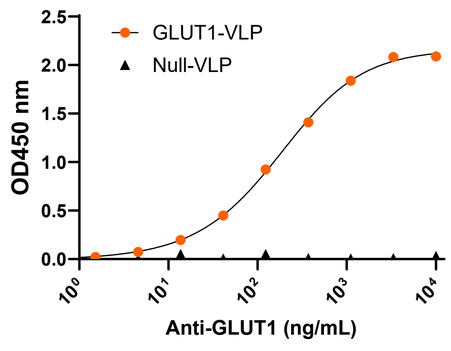
IC-X VLP and ligand binding



The IC-X target on the VLP can bind its ligand specifically and potently, EC50 < 0.03 ug/ml, as measured by ELISA.

6. Transporter GLUT1 (12-TMD) VLP binding to antibody

GLUT1-specific antibody binding

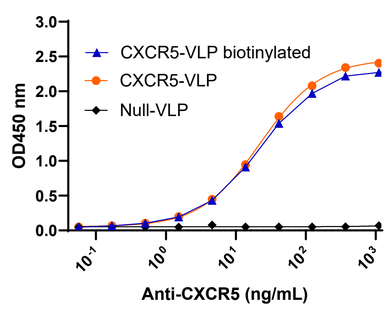


The GLUT1 target on the VLP can bind the target-specific antibody specifically and potently, EC50 < 0.2 ug/ml, as measured by ELISA.

7. Optimized biotinylation does not impact the target bioactivities on VLPs

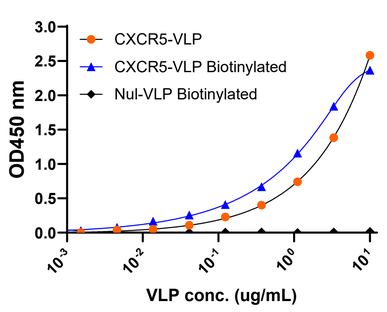
Biotinylated CXCR5-VLP binds specific antibody (A) and ligand CXCL13 (B) similarly to unbiotinylated CXCR5-VLP

A.



The biotinylated CXCR5 VLP can bind the specific antibody very similar to the unbiotinylated CXCR5 VLPs, with , EC50 < 0.02 ug/ml, as measured by ELISA.

B.



The biotinylated CXCR5 VLP can bind the ligand CXCL13 very similar to the unbiotinylated CXCR5 VLPs, with , EC50 < 5 ug/ml, as measured by ELISA.

Conclusions

- Conigen's VLPs displaying multi-pass membrane proteins (4-12 transmembrane, TMD) on the surface in natural lipid bilayer have higher target protein densities resulting in stronger ligand binding.
- The target proteins expressed on the VLP surface have native conformation and are bioactive in binding to their specific antibodies and ligands.
- Example VLPs: 4-TMD Claudin family targets: Claudin 18.2 and Claudin 6; 7-TMD GPCR chemokine receptors: CXCR5, CXCR4 and CX3CR1; 10-TMD ion channel protein: IC-X; and 12-TMD transporter protein GLUT1, can all potently bind to their specific antibodies and ligands if there is any.
- The VLP biotinylation preserves the integrity of the target protein, and the targets biotinylated VLPs, CXCR5 VLP as an example, have very similar binding specificity and potency as the un-biotinylated VLPs.
- The multi-pass membrane proteins (4-12 TMDs) VLP nanoparticles can serve as antigens and immunogens for research and therapeutic discovery.