# Ligand Binding to Membrane Proteins using Lipoparticles

## Membrane Protein Binding Assays

Approximately 50% of therapeutic drugs act on membrane proteins embedded within cell membranes, such as G protein-coupled receptors and ion channels. Surface membrane proteins serve as a functional interface between the cell and its environment, making them desirable pharmaceutical targets. Due to their structure, membrane proteins present a unique set of challenges in the laboratory. Membrane proteins often cross the lipid bilayer multiple times, and are typically unstable when removed from it. This makes them difficult or impossible to isolate and purify in their native structure, and restricts methods by which they can be experimentally manipulated. Conventional binding assays for membrane proteins typically require the use of whole cells or vesicles derived from crude cell membrane preparations. These formats, however, are inconvenient to utilize, are poorly suited to advanced detection devices (e.g. microfluidics), and contain heterogeneous mixtures of proteins that contribute to high experimental variation.

## The Lipoparticle

Lipoparticles are nano-scale structures derived directly from cell membranes, and contain high concentrations of enriched, structurally-intact membrane proteins. Because they are easily manipulated and non-living, they are well-suited for high-throughput screening assays such as conventional radioligand binding, or wash-free assays that use scintillation proximity (SPA) or fluorescence polarization. Their small size (approximately 150 nm) allows them to be assayed in existing and emerging miniaturized and microfluidic platforms Most membrane proteins can be incorporated into Lipoparticles, and membrane protein purities are 10 to 100-fold greater than those of cells or traditional membrane preparations. This translates into significantly enhanced assay sensitivity, specificity, and reproducibility, even with targets that are characteristically resistant to screening procedures due to poor cell expression or toxicity.

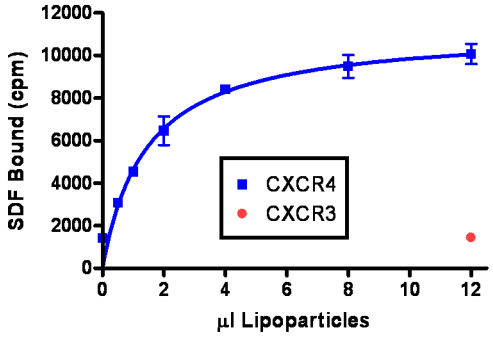


**Figure 1.** Saturation binding indicates binding of SDF-1α to a Lipoparticle preparation containing its cognate receptor CXCR4. The amount of Lipoparticles required to achieve a half-maximal signal for the amount of radioligand added (0.1 nM) was 1.49 µl (0.15 µg). Results shown were performed in duplicate and are representative of two similar experiments.

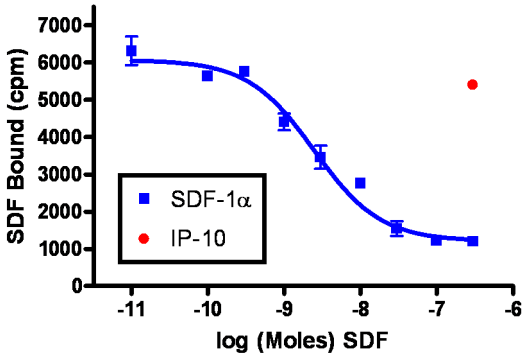


**Figure 2.** Competitive displacement of radioactive SDF-1α from CXCR4 Lipoparticle preparations using unlabeled ligand. The Lipoparticle preparation was estimated to have a functional receptor concentration of 230 pmol/mg total protein. Results shown were performed in duplicate and are representative of two similar experiments.

## Technical Description

The experiments shown here demonstrate the utility of Lipoparticles for routine radioligand binding assays. Lipoparticles containing the chemokine receptor CXCR4 were incubated with the receptor's cognate ligand SDF-1α. Figure 1 shows a typical saturation binding curve generated when increasing quantities of CXCR4-Lipoparticles are mixed with a fixed concentration of radiolabeled SDF-1α. Competitive displacement of the radioactive ligand using unlabeled SDF-1α is shown in Figure 2. Both binding curves are consistent with specific, saturable ligand binding, and indicate that Lipoparticle-incorporated CXCR4 is structurally intact. Non-specific binding is minimal, as indicated by substitution with Lipoparticles expressing the receptor CXCR3 (which does not bind SDF-1α) or by substitution with the chemokine ligand IP-10 (which does not bind CXCR4). From these binding data, the concentration of receptors in the Lipoparticle preparation was determined to be 230 pmol/mg total protein, about 10-100-fold more concentrated than most other sources of membrane protein. Other Lipoparticle preparations have been similarly calculated to contain specific membrane protein concentrations in the range of 50-200 pmol/mg.

## Contact Us

Lipoparticles are produced with customized membrane proteins, and are validated for use with specific applications. Evaluation materials are available for new applications. For more information contact us at:

**215.966.6061**
**info@integralmolecular.com**
**www.integralmolecular.com**