

Immobilization of Membrane Proteins on Beads using Lipoparticles

Bead Technology

Specialized beads provide a mobile substrate to facilitate the separation and manipulation of biomolecules. Agarose, paramagnetic, and polystyrene beads enable attached proteins to be used as highly-pure targets within a number of assay formats, including chromatography, centrifugation, or magnetic separation. Due to their conformational requirements for an intact membrane, integral membrane proteins have been more difficult to adapt to bead technology. The attachment of whole cells or membrane vesicles to beads is limited by size, the fragility of cells and lipid membranes, and the heterogeneity of proteins in these sources. Integral Molecular's Lipoparticle technology enables high concentrations of structurally-intact membrane proteins to be attached to a wide variety of bead types.

The Lipoparticle

Lipoparticles are cell-free nanometer-sized particles engineered to incorporate high concentrations of a target membrane protein. Lipoparticles containing a wide variety of membrane proteins, including GPCRs, single-transmembrane receptors, and ion channels, have been produced, and all proteins have been shown to be structurally intact. Because they are derived from the cell membrane, Lipoparticles can be attached to beads similarly to other membrane structures (e.g. membrane preparations). However, Lipoparticles are stabilized by internal structures and can withstand mechanical manipulations that can damage other sources of membrane proteins. Lipoparticles can be used to reliably and stably attach physiologically-relevant membrane proteins to beads for applications including ligand

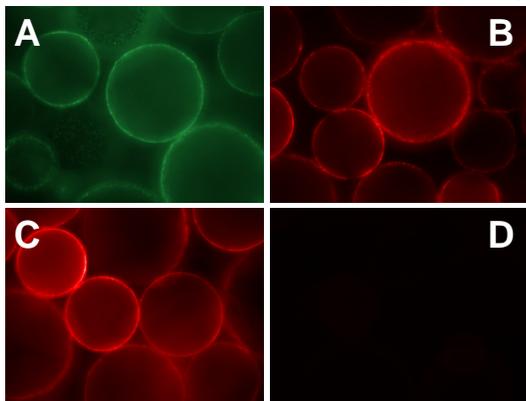


Figure 1. Structurally-intact GPCRs immobilized on WGA agarose beads. (A) Visualization of fluorescent Lipoparticles confirmed intact attachment to beads. Beads were immuno-stained using antibodies reactive to either (B) CXCR4 or (C) CCR5. Antibody binding was visualized using a Cy3 secondary antibody. (D) A non-specific primary antibody demonstrated no significant signal.

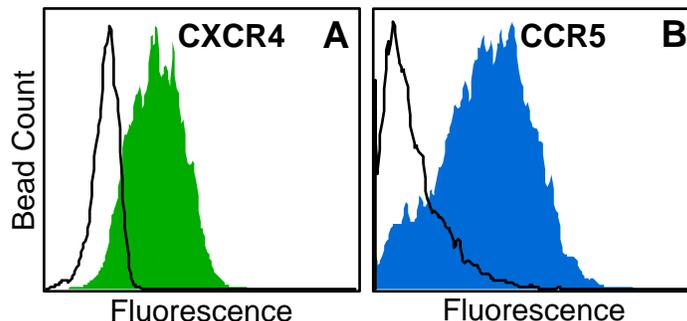


Figure 2. Flow cytometry analysis of Lipoparticle-incorporated GPCRs attached to beads. (A) CXCR4 and (B) CCR5 Lipoparticles were attached to 1 μ m beads, stained, and detected. An Alexa 488-conjugated secondary antibody was used. Controls (black line) included Lipoparticles without any receptor, or receptor Lipoparticles without primary antibody.

fishing, chemical library screening, flow cytometry, column chromatography, immunoprecipitation, and phase display.

Technical Description

Lipoparticles have been attached to a variety of beads for use in different applications. Lipoparticles incorporating either the GPCR CXCR4 or CCR5 were mixed with wheat germ agglutinin (WGA)-coated agarose beads, concentrated by centrifugation, and washed. Binding of intact Lipoparticles to bead surfaces was confirmed by visualizing a green fluorescent tag contained within the Lipoparticle core (Figure 1A). Bound Lipoparticles were then immunostained using conformationally-sensitive anti-CXCR4 or anti-CCR5 antibodies, or a non-specific antibody (Figures 1B-D). The receptor-specific binding of these antibodies indicates that bead-attached GPCRs retain their tertiary structure. A cytoplasmic epitope was not accessible to antibody staining, suggesting that Lipoparticle membranes were not compromised during attachment, washing, or staining procedures (not shown). Lipoparticles have also been attached to paramagnetic polystyrene beads. CXCR4- or CCR5-Lipoparticles were attached to 1 μ m beads coated with a carboxylated glycidyl ether, and the beads were immunostained and analyzed by flow cytometry (Figure 2). Lipoparticle-coated beads were clearly differentiated from controls by fluorescence, indicating the high density of structurally-intact receptors present on each bead.

Contact Us

Lipoparticles are available with customized receptors, and are validated for specific applications. Lipoparticles are provided with protocols for attachment to a variety of substrates. Evaluation materials are available for testing. For more information contact us at:

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