

Sources of Membrane Proteins

Intact cells and membrane preparations have traditionally been useful sources for studying the binding of G protein-coupled receptors (GPCRs) to radiolabeled molecules and for studying their signaling through G protein pathways. Cells and membrane preparations are inexpensive and convenient formats for studying GPCRs, but their use is often limited by impurities and heterogeneity. Cells contain many receptors and complex signaling pathways in addition to those being studied, and must be carefully maintained. Membrane preparations can be contaminated by intracellular membrane structures, and up to half of the membrane proteins in a preparation can be inverted and therefore not useful for analyzing surface receptor functions. A concentrated source of GPCR protein with the convenience of membrane preparations and the reliability of structurally-intact receptors on the cell surface would enable higher quality GPCR assays to be conducted.

The Lipoparticle

Lipoparticles are stable, nanoscale (150 nm diameter) membrane particles derived directly from cells using retroviral structural proteins. They are engineered to incorporate high concentrations of a specific GPCR (or other integral membrane protein) on their membrane surface. Because Lipoparticles are generated through a controlled process that is mediated by a viral structural core protein, they are homogeneous in size, contain highly concentrated populations of target receptor, and are free from intracellular contaminants. Their lipid bilayer is derived directly from the cell surface, so the incorporated receptors are structurally-intact and correctly-oriented within the membrane. Where desired, Lipoparticles have been engineered to reconstitute the G protein components of the GPCR signaling pathway.

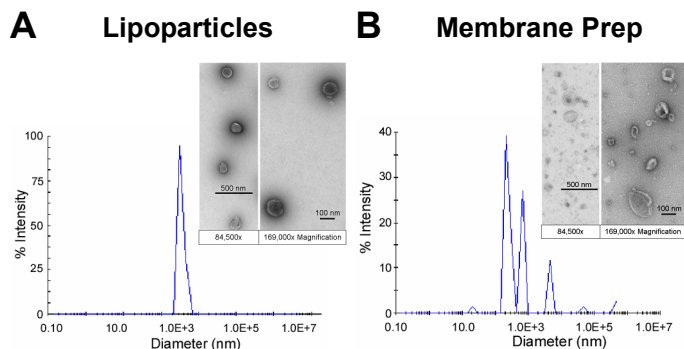


Figure 1. Lipoparticles are more pure than conventional membrane preps. Dynamic light scattering (DLS) profiles and negative-stained electron microscope images for (A) Lipoparticles and (B) membrane preparations, both produced from HEK-293 cells. EM images were taken at 84,500x and 169,000x magnification.

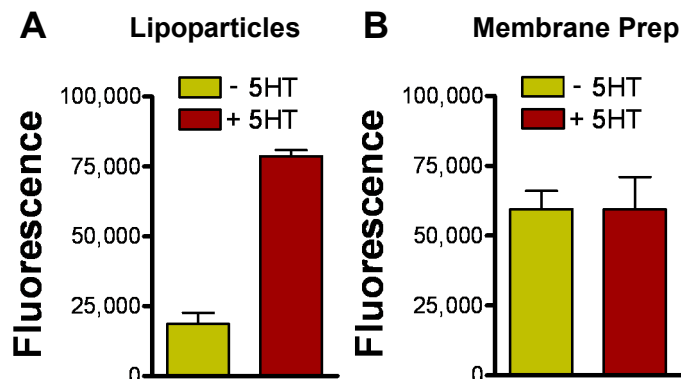


Figure 2. Monitoring GPCR activation using fluorescent probes. (A) Lipoparticles and (B) conventional membrane preps, both produced from HEK-293 cells over-expressing 5HT1A, were monitored in an assay of receptor activation using a fluorescent GTP analog.

Technical Description

Lipoparticles and conventional membrane preparations were directly compared as protein sources for studying the serotonin receptor 5HT1A. Lipoparticles were produced from HEK-293 cells over-expressing the 5HT1A GPCR. Conventional membrane preparations were produced on the same day from a sub-population of these cells using traditional mechanical disruption, fractionation, and centrifugation methods. Dynamic light scattering and direct visualization by electron microscopy revealed that Lipoparticles exhibit substantially greater homogeneity than membrane preparations (Figure 1). Protein and lipid contaminants in membrane preparations can result in unwanted noise and variability in radioligand binding assays. The consequences of these contaminants were evaluated using a receptor signaling assay conducted using a fluorescent GTP analog that fluoresces only when bound to activated G protein. 5HT1A-Lipoparticles exhibited highly specific signaling activity in response to serotonin in this assay (Figure 2). In contrast, the membrane preparation exhibited substantial fluorescence even in the absence of serotonin, most likely due to GTP binding and cleavage by endogenous contaminants. Their homogeneity, purity, and convenience make Lipoparticles an attractive alternative to conventional membrane preparations and live cells, both for binding assays and signaling assays.

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