Attachment of Lipoparticles to the ProteOn XPR36

Optical Biosensor Surfaces

Optical biosensors represent valuable tools in basic and applied research for measuring the affinity, specificity and kinetics of molecular interactions. Biosensor experiments involve immobilizing molecules of interest (ligands) on a chip surface and assessing the binding of interacting molecules (analytes) that are flowed across the chip. For soluble proteins, biosensor methodologies have been well developed. However, this technology has not been widely employed with membrane proteins due to the structural requirements of membrane proteins for an intact lipid bilayer and the difficulty of attaching such structures to biosensor chip surfaces. Whole cells and membrane preparations are too large and heterogeneous for application to biosensors. Other types of lipid-based structures, such as enveloped virus particles and liposomes, can also be problematic to attach due to potential deformation of the lipid bilayer and inactivation of the embedded membrane proteins. Integral Molecular's Lipoparticle technology makes biosensor applications accessible to membrane proteins by presenting them in a format that is readily amenable to surface attachment using validated protocols. In conjunction with the array-based ProteOn biosensor, Lipoparticles enable a convenient platform for screening membrane protein interactions with analytes such as antibodies.

Lipoparticle Attachment

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 membrane protein on their surface at concentrations 10-100 fold higher than those found in cells or membrane preparations. Lipoparticles can be directly attached to biosensor chip surfaces via amine coupling. Alternatively, they can be indirectly attached to biosensor chip surfaces using a capture antibody against native surface proteins on the Lipoparticle surface, or using wheat germ agglutinin (WGA) lectin coupling. All three methods permit reproducible attachment of Lipoparticles without compromising the structural integrity of target membrane proteins. Since the attachment methods are common for all Lipoparticles, an entire array based biosensor chip can be rapidly prepared with a single attachment protocol for the analysis of diverse membrane proteins.

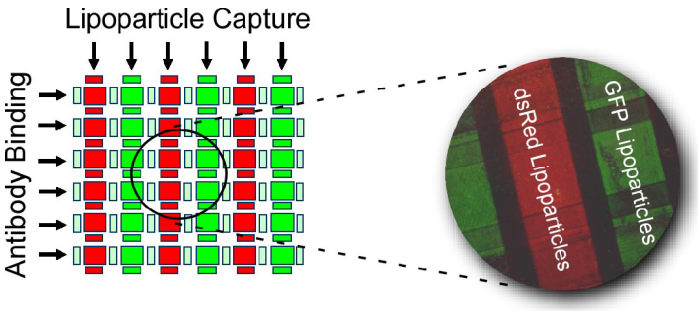

Figure 1. Schematic diagram of a ProteOn chip and fluorescence microscopy image of a GLC chip with immobilized Lipoparticles in verticle channels captured via the JS-81 antibody. Captured Lipoparticles contain either GFP or dsRed fluorescent proteins.

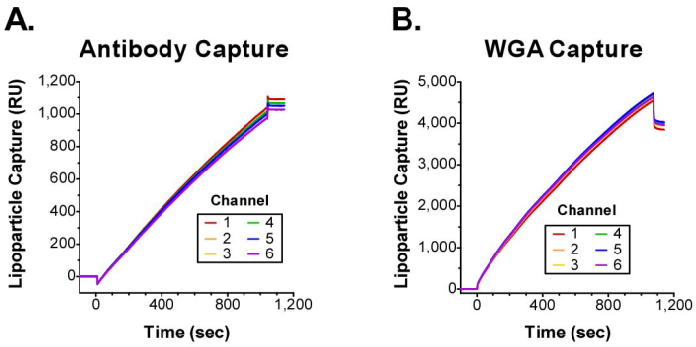

Figure 2. CXCR4 Lipoparticles were captured on a ProteOn GLC chip containing either (**A**) JS-81 capture antibody or (**B**) WGA lectin immobilized on the chip surface. The six sensorgram traces in each figure represent measurements taken at each of the six interaction spots in the channel.

Technical Description

Bio-Rad ProteOn XPR36 sensor chips (GLC, GLM, and GLH) are composed of a modified carboxylated alginate which is easily activated for general amine coupling and amenable to WGA and antibody based capture methods. In **Figure 1**, Lipoparticles containing either green fluorescent protein or dsRed were attached to alternate channels on a ProteOn GLC chip using the antibody JS-81, which recognizes a native protein (CD81) on the surface of Lipoparticles. Lipoparticles were flowed across, and captured to the biosensor chip, then visualized by fluorescence microscopy. **Figure 2** shows sensorgrams measuring real-time capture of Lipoparticle to a GLC chip using either antibody capture or WGA coupling. For each flow channel, similar levels of attachment were obtained for all six interaction spots. Importantly, the membrane proteins within the Lipoparticles retain native conformation after attachment, as measured by binding of conformationally-sensitive antibodies. Antibody capture and WGA coupling are preferred attachment strategies for Lipoparticles since they allow the biosensor chip to be more easily regenerated for re-use.

Contact Us

Lipoparticles are available from Integral Molecular for the study of membrane proteins. The ProteOn XPR36 is available from Bio-Rad Laboratories (www.biorad.com) for the study of protein interactions. For more information contact Integral Molecular at:

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