Rapid Screening of Antibodies against Membrane Proteins using a Biosensor

Biosensor Screening of Antibody Panels

Biosensor screening of panels of antibodies, such as hybridoma supernatants or affinity-matured antibodies, is especially valuable for characterizing antibodies of very high (<0.1 nM) or low (>100 nM) affinity where equilibrium binding assays are difficult or inaccurate. Optical biosensors enable such measurements to be collected in real-time, with high sensitivity, and without the need for fluorescent or radioactive labels. However, biosensors have not been widely used for antibody screening against membrane protein targets, such as GPCRs and ion channels, because of the inherent difficulties of manipulating membrane proteins within microfluidic devices. Integral Molecular's Lipoparticle technology enables rapid biosensor screening of antibodies to test for membrane protein specificity and relative affinity.

The Lipoparticle

Lipoparticles are a novel format for studying membrane proteins that enables their analysis using optical biosensors. Traditional sources of membrane proteins (whole cells or crude membrane preparations) are typically too large or impure to generate high quality biosensor data. Lipoparticles, which are derived directly from cellular plasma membranes, are 150 nm in diameter and contain high concentrations of stable, conformationally-intact membrane proteins at purities up to 100-fold those of cells and membrane preparations. Integral Molecular has developed validated protocols for biosensor analysis of antibodies using Lipoparticles, and has demonstrated their utility with a number of membrane proteins. When used as mobile-phase analytes in biosensor experiments, Lipoparticles enable rapid and reliable biosensor screening of antibodies against membrane protein targets. Binding signals in optical biosensor experiments are enhanced by the Lipoparticle's large size and intrinsic multivalency.

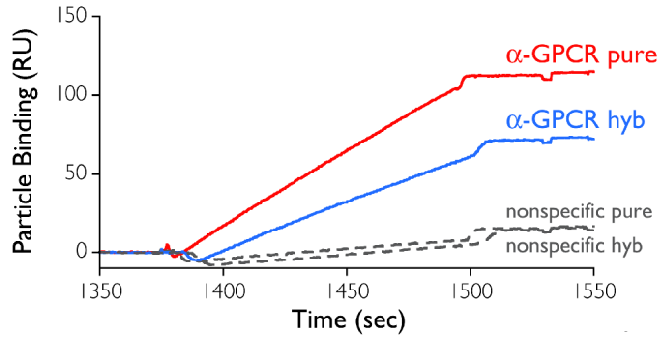

Figure 1. Binding of GPCR-containing Lipoparticles to Specific Antibodies. Purified antibodies (red), unpurified hybridoma supernatants (blue), and non-specific antibodies (gray) were captured onto different flow cells of a Biacore biosensor chip. Lipoparticles containing the GPCR of interest were then flowed over the chip surface. Lipoparticles bound to specific antibodies but not to irrelevant antibodies.

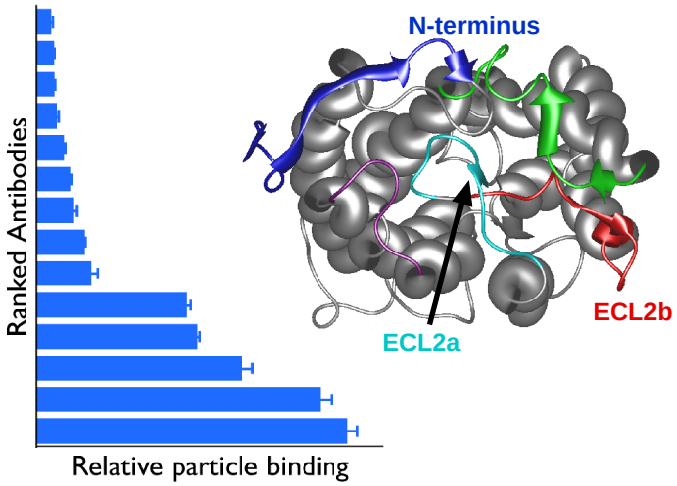

Figure 2. Screening of a Panel of CCR5 Antibodies. Antibodies reactive against diverse epitopes of CCR5, including those regions highlighted on the 3-dimensional model (top-down view), were captured on a biosensor chip. The binding of CCR5-containing Lipoparticles to each of the antibodies was measured in duplicate experiments on a Biacore biosensor.

Technical Description

A panel of monoclonal antibodies (MAbs) directed against the GPCR CCR5, a well-described chemokine receptor, was evaluated for specificity and relative affinity. The antibodies included well-characterized and broadly neutralizing MAbs, conformationally-sensitive MAbs, MAbs with diverse epitope specificities, and unreactive control MAbs. MAbs were arrayed in 96-well microplates and sequentially captured to similar levels on a secondary-antibody chip surface. Lipoparticles incorporating CCR5 were then injected across all flow-cells of the chip to test for MAb binding and to measure relative affinity (Figure 1). All MAbs were readily and reliably ranked by the strength of the Lipoparticle binding interaction to the chip surface (Figure 2). Rankings were determined to be accurate from the literature and from more detailed kinetic analyses. Hybridoma supernatants and native enveloped viruses have also been screened using this strategy. The same approach can enable the screening of binding interactions between membrane proteins and any molecule that can be attached to a biosensor surface.

Contact Us

Biosensor analyses of membrane protein interaction kinetics are provided to customers on a fee-for-service basis, which includes customized Lipoparticle production, data collection, and kinetic analyses. For more information contact us at:

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