

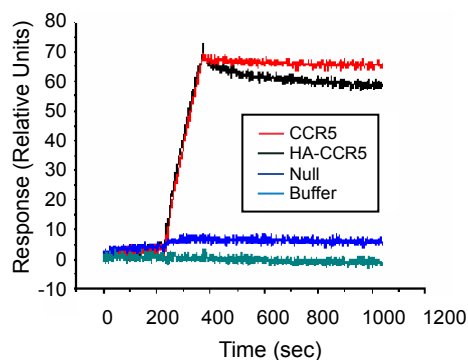
# Kinetic Analysis of Membrane Protein MAbs using an Array-based Biosensor

## Array-based Optical Biosensors

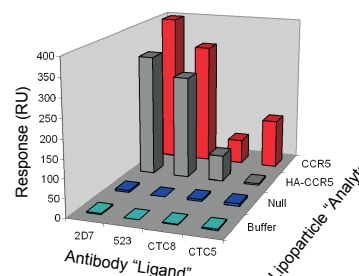
Optical biosensors allow for real-time detection of interactions between biological molecules, such as proteins and their ligands. In addition to measuring overall binding strength (affinity,  $K_D$ ), optical biosensors can also measure individual molecular association and dissociation rates (binding kinetics,  $k_{on}$  and  $k_{off}$ ). Because optical biosensors are sensitive to interactions over a wide affinity range and do not require labels for detection, they have increasingly been incorporated into scientific investigations. While conventional optical biosensors are typically limited to a small number (e.g. 4) of binding reactions, array-based systems have increased the analytical capacity for measuring protein interactions. Bio-Rad's ProteOn XPR36 optical biosensor platform comprises intersecting channels in which up to six captured ligands can be interrogated by up to six flowed analytes, allowing up to 36 analyses within a single experiment. Multichannel biosensors are ideal for higher throughput binding experiments, such as screening of antibodies for therapeutic or diagnostic applications.

## Lipoparticles as Biosensor Reagents

Characterizing antibodies for their ability to bind integral membrane proteins, such as GPCRs and ion channels, is typically complicated by the difficulty of manipulating membrane proteins for biosensor analyses. Whole cells are poorly suited to microfluidic devices, and crude membrane preparations are heterogeneous and impure, limiting the quality of binding data that they can generate in highly-sensitive detection systems. Integral Molecular's Lipoparticles are novel membrane protein reagents that are compatible with biosensor microfluidic systems. Lipoparticles are 150 nm particles composed of a cellular lipid bilayer wrapped around a non-infectious viral protein core. The Lipoparticle membrane contains high concentrations of a target membrane protein of interest.



**Figure 1. Sensorgrams indicating specific binding of Lipoparticles to captured MAbs.** Responses were observed in channels challenged with CCR5- or HA-CCR5-Lipoparticles, but not in those challenged with null-Lipoparticles or with buffer alone. Typical results for one captured target MAb are shown.



**Figure 2. Multiple MAbs can be simultaneously screened against a number of Lipoparticle analytes.** Each captured antibody was tested for specificity and relative affinity against specific and non-specific membrane proteins presented within Lipoparticles.

Because membrane proteins are captured directly from cell surfaces onto Lipoparticles, they retain their native conformation. Lipoparticles enable membrane proteins to be manipulated in fluid suspension, are highly homogeneous, and contain membrane proteins enriched approximately 10-100-fold in purity. Lipoparticles incorporating a range of membrane proteins have been validated for use in optical biosensors as both chip-immobilized "targets" and as mobile-phase "analytes".

## Technical Description

To analyze membrane protein interaction kinetics using the Bio-Rad ProteOn XPR36 (Bio-Rad Laboratories, Hercules, CA), a panel of four monoclonal antibodies (MAbs) of known reactivity were simultaneously screened against the chemokine receptor CCR5. Capture antibody was amine coupled to a ProteOn GLC Sensor chip, and the CCR5 MAbs were captured on parallel chip channels. Lipoparticles incorporating either wild type CCR5 or an HA-tagged CCR5 recombinant protein, null Lipoparticles containing no specific membrane protein, or buffer alone were simultaneously flowed across the chip in orthogonal chip channels. Individual sensorgrams (Figure 1) clearly indicated MAb binding in channels containing CCR5 or HA-CCR5 Lipoparticles, but not in channels containing null Lipoparticles or buffer alone. From these biosensor responses, the binding of high affinity MAbs could be quantitatively differentiated from that of low affinity MAbs. Masking of the N-terminus structure by the HA tag was also observed, as expected (Figure 2). These results demonstrate the suitability of Lipoparticle reagents in multichannel optical biosensors for scientific, diagnostic, or therapeutic development.

## 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](http://www.biorad.com)) for the study of protein interactions. For more information contact Integral Molecular at:

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