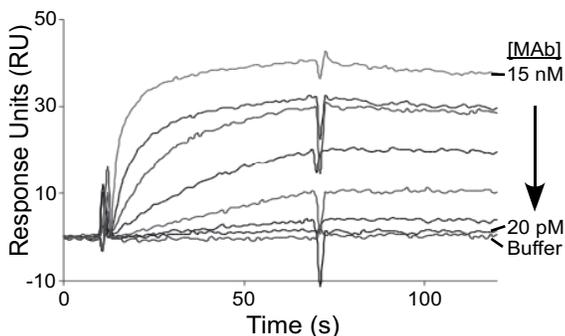


## Optical Biosensors

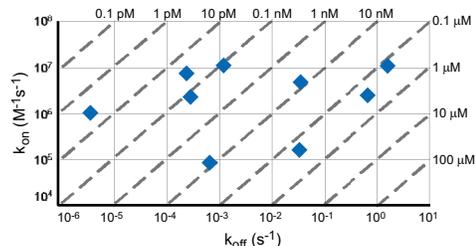
The optical biosensor is an established technology for measuring interactions between biological molecules in real-time, with high sensitivity, and without the need for fluorescent or radioactive labels. Unlike conventional equilibrium binding assays, which typically yield only static, single-point measurements (generally equilibrium binding affinity,  $K_D$ ), optical biosensors also provide useful kinetic binding data. This is important because molecular 'on rates' ( $k_{on}$ ) and 'off rates' ( $k_{off}$ ), not just equilibrium affinity, can be critical determinants of the quality of ligand-receptor and drug interactions. Biosensors are especially valuable for measuring interactions with extremely high (<0.1 nM) or low (>100 nM) affinities, where equilibrium binding assays are difficult or inaccurate. Applications of optical biosensors include kinetic analysis, diagnostic detection, structure-function correlation, ligand fishing, and phage selection. These applications have previously been restricted to soluble (non-membrane-bound) binding partners, but Integral Molecular's Lipoparticle technology is making these applications accessible to membrane proteins. Biosensors usually contain a chip surface upon which molecules of interest ("ligands") are immobilized. The binding of interactive molecules ("analytes") flowed across the captured ligand is detected as a change in refracted light (e.g. surface plasmon resonance). Lipoparticles allow structurally intact membrane proteins to be used as either the ligand or the analyte.

## The Lipoparticle

Membrane proteins require a lipid membrane to maintain structural integrity, making them difficult to manipulate experimentally, and restricting their use with optical biosensors. Lipoparticles are a novel format for presenting and manipulating transmembrane proteins that enable their application to optical biosensors. Traditional formats for working with membrane proteins (whole cells



**Figure 1.** Biacore biosensor sensorgram for an anti-CXCR4 antibody. Binding curves are concentration dependent (3-fold serial dilutions of the antibody). Concentrations of antibody as low as 20 pM could be detected.



**Figure 2.**  $k_{on}$  vs.  $k_{off}$ . Measurements on the same diagonal possess the same  $K_D$  despite having different  $k_{on}$  and  $k_{off}$  values, demonstrating the utility of real-time measurements for defining molecular interactions.

or cell membrane preparations) are typically too impure or heterogeneous to generate high quality biosensor data. Lipoparticles, which are derived directly from cell membranes, are approximately 150 nm in diameter, and contain high concentrations of stable membrane proteins at purities up to 100-fold greater than those of cells or cell membrane preparations. Integral Molecular has developed optimized protocols for biosensor analysis using Lipoparticles, and has demonstrated their utility with a number of membrane proteins.

## Technical Description

Biosensor results generated using Lipoparticles and a Biacore optical biosensor are shown in Figures 1 and 2. Lipoparticles incorporating the chemokine receptors CXCR4 or CCR5 were immobilized on a biosensor chip. Nine monoclonal antibodies recognizing mostly conformation-dependent epitopes on the receptors were flowed across the chip. Figure 1 demonstrates typical binding curves (sensorgrams) generated at various antibody concentrations. Antibody binding is concentration dependent and saturable, demonstrating the specificity of the interaction. Figure 2 shows a plot of apparent (bivalent)  $k_{on}$  vs.  $k_{off}$  for all nine antibodies. The data demonstrates that antibodies with similar affinities can possess different  $k_{on}$  and  $k_{off}$  values, emphasizing the value of real-time kinetic data generated by biosensors. Membrane protein interactions can also be measured when Lipoparticles are flowed across biosensor surfaces containing immobilized antibody. Biosensors are a primary means by which kinetic values for antibody panels and other ligand-receptor pairs are derived. With Lipoparticles, this technology can now also be applied to integral membrane proteins.

## 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. Lipoparticles are also available on a custom basis. For more information contact us at:

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