One-Shot Kinetic Analysis of Membrane Protein Interactions

Biosensor Analysis of Protein Interactions

Optical biosensors are used to measure the overall binding affinity (K_D) as well as real-time molecular association and dissociation rates (k_a and k_d) of protein interactions. Conventional biosensors can typically measure only a small number of binding interactions, while array-based systems such as Bio-Rad's ProteOn XPR36 have markedly increased the capacity for measuring protein binding kinetics. Instead of using multiple attachment-regeneration cycles to assess binding kinetics with different analyte concentrations, the ProteOn One-Shot approach offers the ability to monitor up to 36 different molecular interactions simultaneously using six different ligands and six different analyte concentrations. Kinetic parameters such as k_a, k_d and K_D can then be quickly calculated using One-Shot kinetic software. Array-based biosensors are ideal for high-throughput binding applications such as screening antibodies for therapeutic or diagnostic applications, but have been difficult to adapt to integral membrane proteins. Integral Molecular's Lipoparticle technology enables biosensor applications with membrane proteins, expanding the ability to study this therapeutically important class of targets.

The Lipoparticle

Biophysical manipulation of membrane proteins is challenging due to their requirement for an intact lipid bilayer, limiting biosensor analyses of these important proteins. Lipoparticles provide a novel strategy for presenting membrane proteins in an essentially soluble format. Lipoparticles are stable, nanoscale (150 nm) virus-like particles derived directly from cellular plasma membranes that contain high concentrations of conformationally-intact membrane proteins, up to 100-fold higher in purity compared to cells or membrane preparations. Receptor-specific Lipoparticles can be attached to biosensor chip surfaces and used as ligands for kinetic binding analyses. When used in conjunction with ProteOn technology, Lipoparticles enable rapid and reliable biosensor screening of antibodies against membrane protein targets, allowing up to six different antibody concentrations to be analyzed simultaneously.

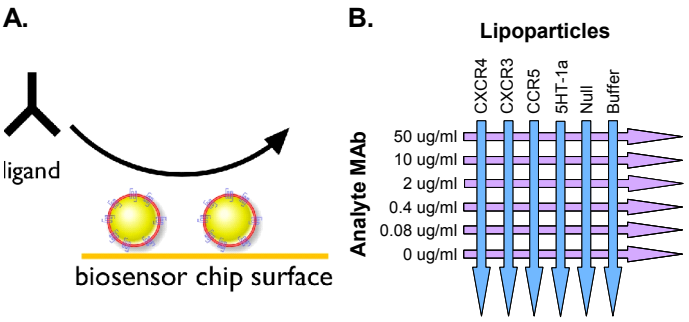

Figure 1. A. Use of Lipoparticles on the ProteOn XPR36 Biosensor. Lipoparticles with membrane proteins of interest are immobilized using a capture antibody on the chip surface, and monoclonal antibody (MAb) analytes are flowed over the attached particles. **B. Proteon chip setup for a One-Shot Kinetic assay.** Vertical (ligand) channels are derivatized with the capture antibody. Different Lipoparticles are then captured on each channel. A dilution series of antibody is then flowed through the horizontal (analyte) channels.

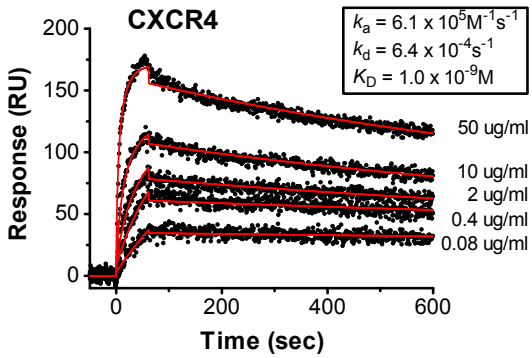

Figure 2. Extraction of kinetic parameters. ProteOn sensorgrams demonstrate binding association and dissociation of an α-CXCR4 antibody to CXCR4 Lipoparticles. An antibody concentration range of 50 to 0.08 ug/ml was used in this One-Shot Kinetic assay. Specific responses are calculated by subtracting the responses to Null Lipoparticles on control flow cells.

Technical Description

To evaluate the specificity and relative affinity of MAbs against various membrane proteins, Lipoparticles containing a range of membrane proteins were immobilized on a multichannel ProteOn XPR36 biosensor chip. An illustration of the Lipoparticles immobilized on the chip surface is shown in **Figure 1A**, and an example chip setup is shown in **Figure 1B**. In a single channel containing immobilized Lipoparticles, interactions with six different concentrations of analyte can be simultaneously measured, resulting in a complete kinetic profile. The array setup of the ProteOn allows six different membrane proteins to be similarly analyzed in a simultaneous fashion. **Figure 2** shows typical binding curves generated with CXCR4-containing Lipoparticles immobilized on a chip and analyzed with different concentrations of an α-CXCR4 antibody. Upon fitting the binding curve for each concentration of antibody, kinetic parameters such as k_a and k_d were determined.

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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