Kinetic Analyses of Binding to Membrane Protein Variants

Array-Based Biosensors

Array-based biosensors such as the Bio-Rad ProteOn XPR36 are particularly well-suited to studying kinetic differences between variants of a target protein. Due to a unique design that facilitates the simultaneous kinetic analysis of up to six protein variants (ligands) with six analytes of interest (**Figure 1**), mutants of a target protein can be compared to determine the effects of specific amino acid changes on affinity, association and dissociation. Integral Molecular's Lipoparticle technology makes these biosensor applications accessible to membrane proteins by presenting them in a nanoscale format within the context of a cell-derived lipid bilayer.

Lipoparticles

Lipoparticles are a novel format for presenting and manipulating transmembrane proteins that enable their use with optical biosensors. Lipoparticles are stable, nanoscale (150 nm) 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. Lipoparticles can be engineered to incorporate virtually any membrane protein including GPCRs and ion channels, and likewise can incorporate specific mutant versions of these proteins. Lipoparticles can be easily attached to biosensor chips, allowing them to be used as ligands for kinetic binding analyses with soluble analytes.

Technical Description

The contributions of specific CXCR4 epitope residues to the binding kinetics of the 12G5 monoclonal antibody (MAb), were assessed by creating a panel of Lipoparticles containing point mutations (E179V, D181V, D182V, R183G, and D187V). These Lipoparticles were immobilized on a ProteOn GLC chip for the simultaneous assessment of binding kinetics. In some cases, disruption of epitope residues such as D181 led to marked changes in the binding profile with 12G5 (**Figure 2**), whereas other epitope mutations had minimal effects. Sensorgrams for all mutants were curve fitted to determine k_a, k_d, and K_D values as shown in an isoaffinity plot (**Figure 3**). Overall, these data reveal that many of the charged contact residues studied are critical for the rate of association (k_a) of 12G5 with CXCR4, but are less critical to the rate of dissociation (k_d), implying the importance of long range electrostatic steering interactions for the initial phase of antibody binding. Understanding the energetic contributions of amino acids that constitute MAb binding sites within receptors is important for developing structural models of MAb-receptor interactions, and for the development of more effective therapeutic antibodies.

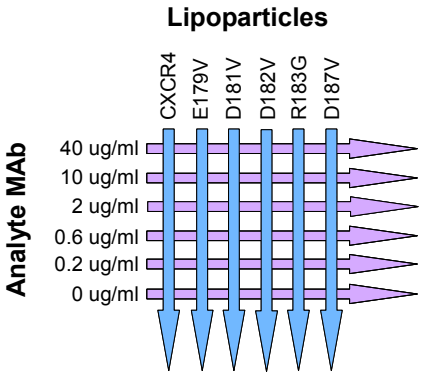

Figure 1. ProteOn chip setup. Lipoparticles containing six different target protein variants can be immobilized on a chip. An experimental setup studying six variants of CXCR4 Lipoparticles is shown here.

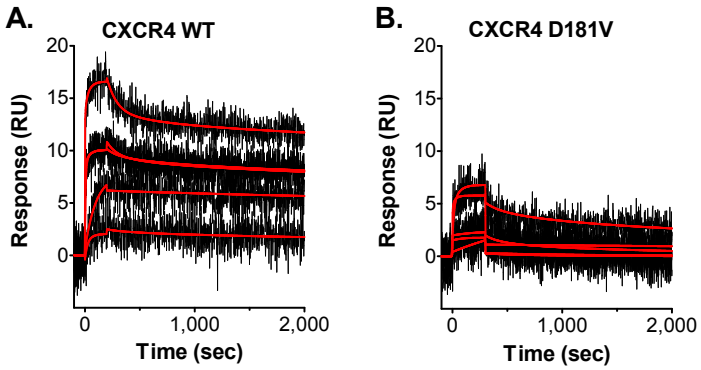

Figure 2. Sensorgrams showing kinetic analysis of wild type (WT) and mutant CXCR4. Binding kinetics of MAb 12G5 to (**A**) CXCR4 WT and (**B**) D181V Lipoparticles were assessed using One-Shot Kinetic experiments. 12G5 bound to WT CXCR4 with high affinity, but bound to the D181V variant of CXCR4 with much lower affinity. Each sensorgram trace represents a 4-fold dilution series of 12G5 antibody (40 µg/ml - 0.2 µg/ml).

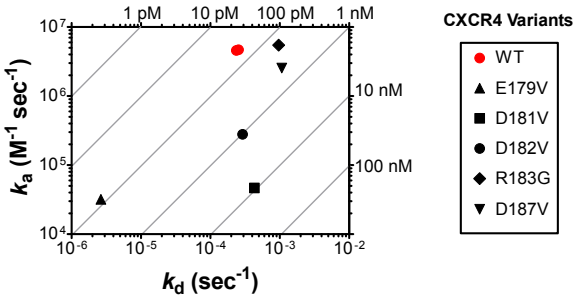

Figure 3. Isoaffinity plot of 12G5 MAb binding to various CXCR4 mutants. Binding sensorgrams from biosensor experiments were curve fitted using One-Shot Kinetics to calculate rate constants k_a and k_d. Downward and rightward shifts represent lower affinity interactions, whereas points on the same line have the same overall affinity (K_D).

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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