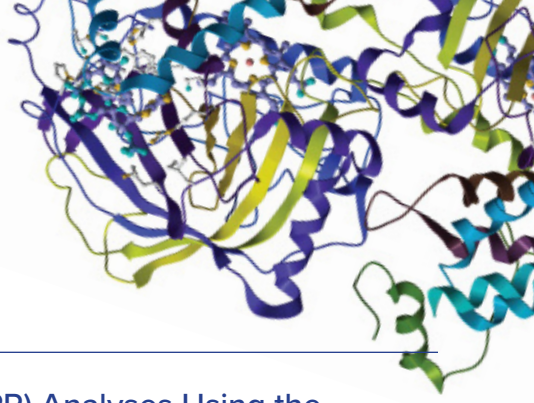

Reichert SPR Small Molecule Analysis

95 Daltons and 201 Daltons Analyte Surface Plasmon Resonance (SPR) Analyses Using the Reichert SR7500DC and Carboxymethyl Dextran Slides

This application note showcases both the very low noise of the SR7500DC Dual Channel SPR system and the utility of Reichert's carboxymethyl dextran slide, CMD500k, for use with small molecule interactions. The interaction between two small molecule inhibitors, methanesulfonamide (95 daltons) and 4-carboxybenzenesulfonamide (4-CBS) (201.2 daltons), and the enzyme carbonic anhydrase (CA II) is studied. Since carboxymethyl dextran sensor chips were first introduced into the marketplace about 20 years ago, they have become the industry standard for obtaining SPR data. They are used to reduce non-specific binding and increase sensitivity. The capture layer of the Reichert CMD500k sensor chip (catalog number 13206066) is a 500,000 dalton carboxymethyl dextran hydrogel coupled to a self-assembled monolayer formed on the gold surface. The combination of the SR7500DC System and the CMD 500k sensor chip yields excellent results for these interactions.

Experimental

The experimental conditions are summarized in the following table:

Ligand	Analyte	Analyte Concentrations	Association Time	Dissociation Time
CA II	Methanesultfonamide	1066, 533, 266, 133 and 66.6 µM	1 min	1 min
CA II	4-CBS	5, 2.5, 1.25, 0.625, 0.3125 and 0.156 µM	1 min	1.5 min

Results

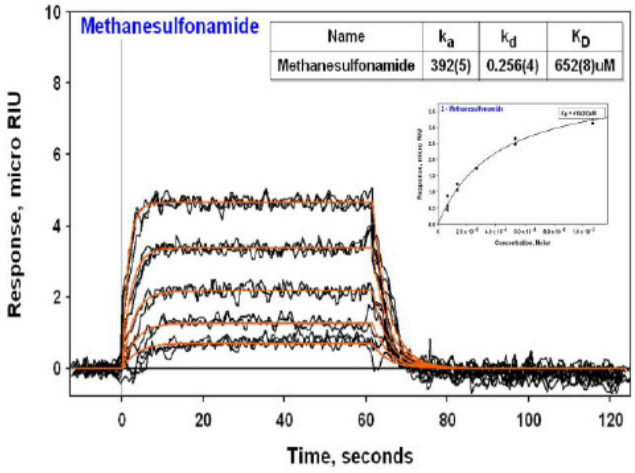

FIGURE 1

Target 1: Methanesulfonamide (95 Da) binding to CA II
About 6,000 mRIU of carbonic anhydrase II was amine coupled to the dextran surface and then the binding of each inhibitor was followed over a series of concentrations (see table).The binding of the low molecular weight inhibitor, methansulfonamide, generates small but very detectable responses owing to the high sensitivity of the SR7500DC. The kinetic fit from Scrubber (Biologic Software) yields an equilibrium dissociation constant (KD) of 652 µM, while the Langmuir isotherm (inset) yields a KD of 410 µM. Both values are within the range of values previously published by other researchers.

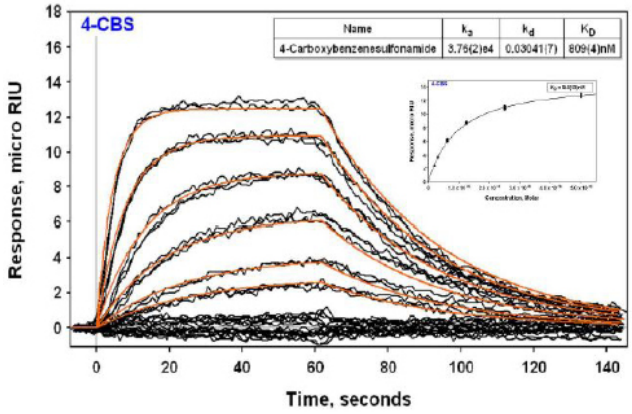

FIGURE 2

Target 2: 4-CBS (201 Da) binding to CA II
4-carboxybenzenesulfonamide binding to carbonic anhydrase II for a series of concentrations (see table) is shown here. Each concentration was injected at least in duplicate. The values obtained for the equilibrium dissociation constant (KD) are 809 nM for the kinetic fit from Scrubber (Biologic Software) and 940 nM for the Langmuir isotherm (inset). Both are within the range of values published in the literature for this binding pair using SPR and isothermal titration calorimetry (ITC).

Learn more about how Reichert pushes the limits of detection and sensitivity in label-free interaction. To improve the quality of your protein interaction research, visit www.ReichertSPR.com

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