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Application Note 2

Small Molecule Assay: 4-Carboxybenzenesulfonamide (201 Da) Binding to Carbonic Anhydrase II

Carbonic anhydrase II (CAII) is an enzyme that catalyzes the reversible hydration of carbon dioxide to form bicarbonate with the release of a proton. CAII has important physiological functions such as pH regulation, bicarbonate metabolism and control of intracellular osmotic pressure.[1] CAII activity is strongly inhibited by a variety of aromatic and heterocyclic sulfonamides. This application note presents the SPR binding experiment between CAII and an inhibitor, 4-carboxybenzenesulfonamide (4-CBS); a small molecule with a molecular weight of 201 Da.

Experimental

The experimental conditions for this assay are summarized below:

Ligand	Analyte	Analyte Concentrations	Association Time	Dissociation Time	Regeneration Solution
CAII	4-CBS	20, 6.7, 2.2, 0.74, 0.25, 0.082 µM	1 min	3 min	None

Results

Figure 1 presents the kinetic results from this small molecule binding experiment. The inset represents the Langmuir binding isotherm where the equilibrium binding response is plotted as a function of concentration. Each concentration is injected at least twice to verify reproducibility. The kinetic data is fit to a simple bimolecular model using Scrubber (Biologic Software) (red lines) and the equilibrium data in the inset of **Figure 1** is fit to a Langmuir binding isotherm model (solid line). This small molecule binding experiment was carried out on four separate occasions and **Table 1** summarizes the results.

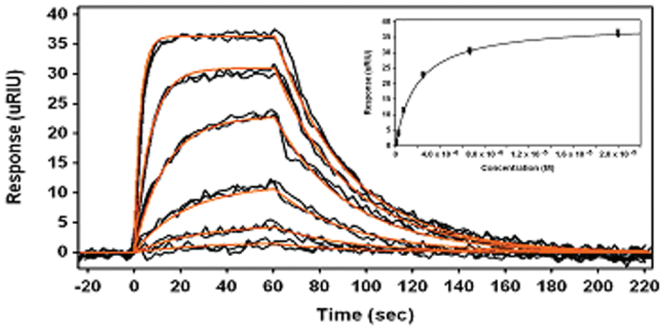

Figure 1: Normalized response versus time plots of 4-CBS binding to CAII fit (red lines) to a simple bimolecular model in Scrubber. The inset is the corresponding Langmuir binding isotherm.

Table 1 presents the association and dissociation rate constants determined from fits to a simple bimolecular model in Scrubber along with the equilibrium dissociation constants (K_D) calculated from the kinetic and equilibrium data, respectively. The results show that the system is highly reproducible and the K_D values determined through the kinetic and equilibrium analysis are in very good agreement with each other and correlate very well with that reported in the literature.[2]

Table 1: Summary of the results from four separate experiments

	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (µM) (Kinetic)	K_D (µM) (Equilibrium)
Run 1	$2.9e^4$	$2.9e^{-2}$	0.96	1.2
Run 2	$2.8e^4$	$2.9e^{-2}$	1.03	1.2
Run 3	$3.2e^4$	$3.2e^{-2}$	1.01	1.1
Run 4	$3.6e^4$	$4.2e^{-2}$	1.19	1.2

[1] Maren, T.H.; Conroy, C.W. *J. Biol. Chem.* **1993**, *268*, 26233.
[2] Papalia, *et. al. Anal. Biochem.* **2006**, *359*, 94.

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