bioanalytics xantec

Application Note 02

Small Molecule Binding Reproducibility

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

Results

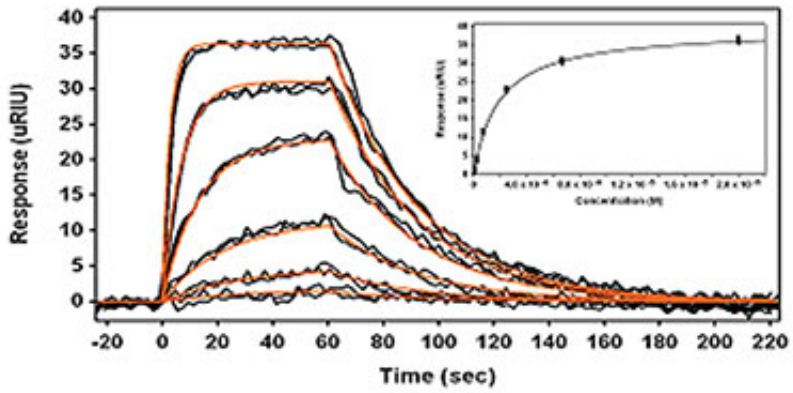

Figure 1 presents the kinetic results from this small molecule binding experiment. The inset represents the Langmuir binding isotherm where the equilibrium binding response is plotted as a function of concentration. Each concentration is injected at least twice to verify reproducibility. The kinetic 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.

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

Table 1 presents the association and dissociation rate binding isotherm. 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.

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