



Application Note 3 Thermodynamic Investigation of an Enzyme-Inhibitor Pair

Understanding the thermodynamics of a system gives valuable insight into the mechanism and forces leading to an interaction. Reichert's SR7000DC system provides the opportunity to acquire thermodynamic data for molecular interactions of interest owing to its precise temperature control from 10 °C below ambient to 90 °C. This application note utilizes the SR7000DC system to characterize the thermodynamics of a small molecule inhibitor-enzyme pair, specifically carbonic anhydrase II (CAII) and 4-carboxybenzenesulfonamide (4-CBS). The binding constants of this interaction are measured with respect to temperature to thermodynamically profile this interaction.

Experimental

The experimental conditions for this assay are summarized below:

Ligand	Analyte	Analyte Concentrations	Temperatures	Regeneration Solution
CAII	4-CBS	20, 6.7, 2.2, 0.74, 0.25, 0.082 μM	15, 20, 25, 30, 35 °C	None

Results

Figure 1 presents the temperature-dependent response curves of 4-CBS binding to CAII at 20 °C (blue lines) and 35 °C (red lines). The results indicate that temperature has a drastic effect on the profile of the response curves. Specifically, the association and dissociation rate constants increase with temperature. To quantify the change in rates, the data at each temperature was fit to a simple bimolecular model using Scrubber (Biologic Software) to determine the rate constants and the equilibrium dissociation rate constants.

Figure 2 presents a van't Hoff analysis of the data by plotting $\ln K_D$ versus $1/T$. The data fits well to a linear regression model ($r^2 = 0.989$), thus the thermodynamic parameter ΔH can be determined directly from the non-integrated form of the van't Hoff equation. In this case, the slope is $\Delta H/R$. ΔH is determined to be -6.0 kcal/mol for this inhibitor-enzyme pair.

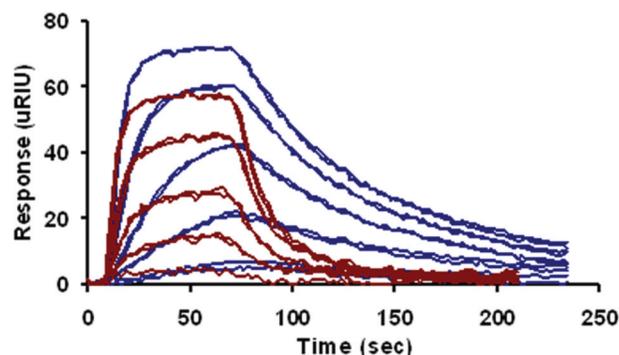


Figure 1: Temperature-dependent response curves of 4-CBS binding to CAII at 20 °C (blue lines) and 35 °C (red lines).

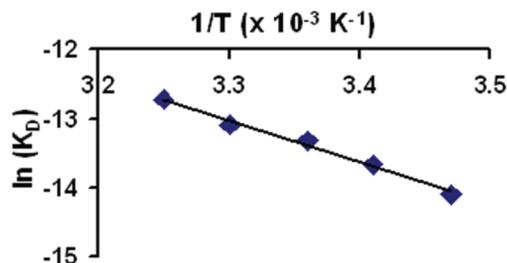


Figure 2: van't Hoff Plot of the Thermodynamic Data from 4-CBS binding to CAII.

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Corporate Headquarters

Reichert Technologies, Inc.
3362 Walden Avenue
Buffalo, New York 14043 USA
Tel. +1 716-686-4500, Fax. +1 716-686-4555
Toll Free USA 1-888-849-8955
Life Sciences: +1 716-686-4522
E-mail: reichertspr.lifesciences@ametek.com

European Headquarters

Reichert Instruments GmbH
Hubertusstrasse 2
D-82229 Seefeld, Germany
Tel. +49 8152 99 3530
Fax. +49 8152 99 35311