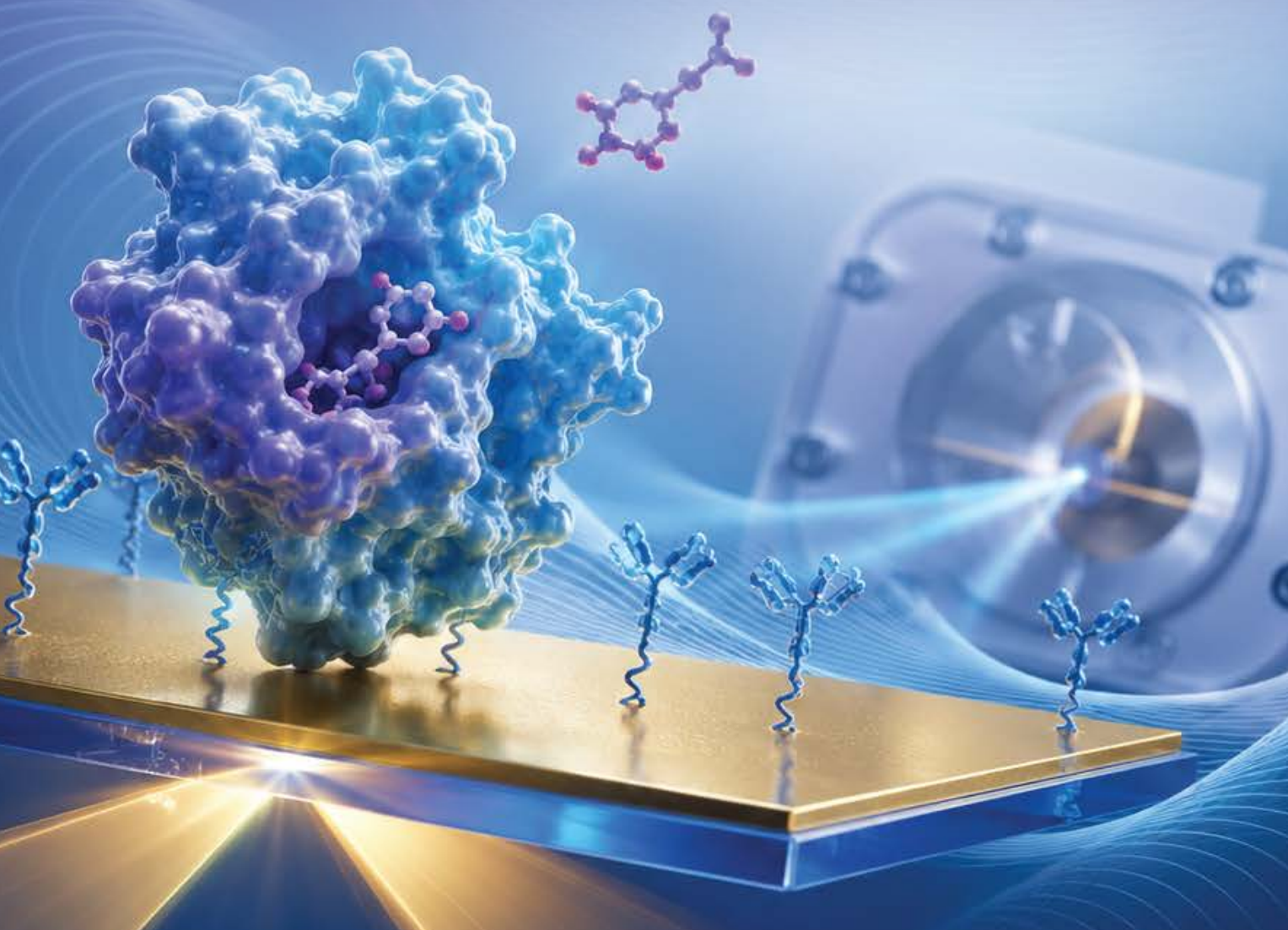


Thermodynamic Investigation of an Enzyme-Inhibitor Pair with Reichert Surface Plasmon Resonance



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.

Keywords or phrases:

Surface Plasmon Resonance (SPR), enzyme-inhibitor interaction, carbonic anhydrase II (CAII), binding kinetics, thermodynamic analysis, van't Hoff plot, Reichert SPR

INTRODUCTION

Thermodynamic profiling of biomolecular interactions plays a critical role in deciphering the balance of forces that govern binding specificity and stability. Beyond affinity, parameters such as enthalpy and temperature dependence provide a more nuanced view of interaction mechanisms, particularly in enzyme-inhibitor systems where subtle energetic shifts can influence function and efficacy. In this context, temperature-resolved kinetic measurements offer a powerful route to connect observable binding behaviour with underlying thermodynamic drivers.

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

Temperatures: 15, 20, 25, 30, 35 °C

Regeneration Solution: 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.

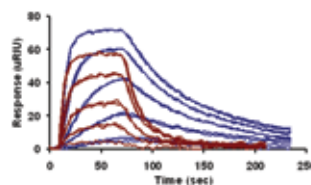


Figure 1: Temperature-dependent response curves of 4-CBS binding to CAII at 20 °C (blue lines) and 35 °C (red lines).

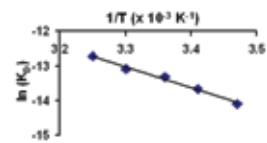


Figure 2: van't Hoff Plot of the Thermodynamic Data from 4-CBS binding to CAII.



Surface Plasmon Resonance