Small Molecule Detection by Surface Plasmon Resonance (SPR)

A major advantage of SPR technology is that the detection does not require the analyte to be labeled, such as with a fluorescent molecule or a redox-active tag. This is because SPR directly detects changes in refractive index resulting from changes in mass at the sensor chip surface. For researchers interested in pharmacology and pharmacokinetics or in general pharmaceutical research or drug discovery, this capability of label-free detection is particularly attractive.

Figure 1 shows binding of acetazolamide to carbonic anhydrase II (CAII), which is an enzyme that rapidly converts carbon dioxide and water to bicarbonate (with proton as a co-product). Carbonic anhydrase inhibitors are a class of pharmaceuticals that inhibit the activity of carbonic anhydrases. Clinically, these inhibitors have been used as antiglaucoma agents to alleviate mountain sickness and to manage neurological disorders.[1] Acetazolamide (molecular weight = 222.22 Da) is one of the commonly used carbonic anhydrase inhibitors. The binding rate constants and affinity value obtained from the kinetics analysis (orange lines) of the SPR binding curves (black lines) are shown in the inset of Figure 1.

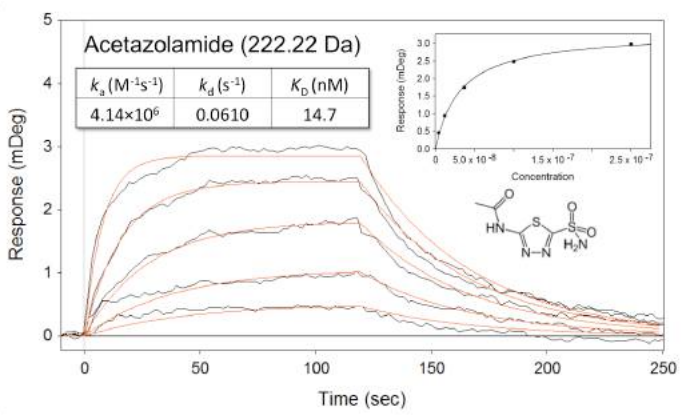

FIG. 1 Binding curves (black) and simulation results (orange) of acetazolamide to pre-immobilized CAII. The acetazolamide concentrations (from bottom to top) are 4.1, 12.3, 37.0, 100.0, and 250.0 nM. The data were fitted with a 1:1 binding model using the Langmuir isotherm. The inset is a table showing the association rate (k_a), dissociation rate (k_d), and affinity binding (K_D) constants. The molecular structure and weight of acetazolamide are also shown.

Excellent fits were achieved and the kinetic and binding parameters in the table are in good agreement with published results. The feasibility for small molecule detection can be attributed to the high sensitivity and low noise of BI SPR detection technology, the fine precision and stability of its BI-DirectFlow™ sampler delivery system, and the excellent performance of BI sensor chips having high loading capacity with low non-specific adsorption.

This note demonstrates that SPR can be used to study the binding interactions of small molecules to proteins. SPR for small molecule detection is a powerful technique that can be used in applications such as screening drug candidates to combat cancers, alleviate the symptom of neurological disorders, and discover antifungal and antibacterial agents.

References:

[1] Supuran CT, Scozzafava A, Casini A, *Med Res Rev* **23** (2): 146–89.