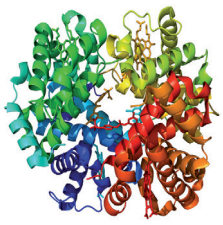
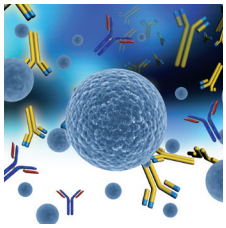
HORIBA Scientific

Surface Plasmon Resonance imaging

The Detection of an Ultralow Molecular Weight Enzyme Inhibitor using the XelPleX™ system





Application Note
Biomedical
SPRi AN38

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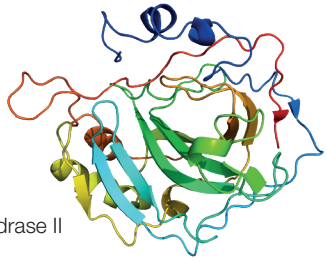
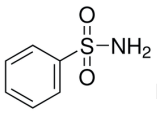
Detection of low molecular weight molecules can be useful especially for pharmaceutical and agri-food applications. This application note is focused on the detection of a 157 Dalton molecule, the benzenesulfonamide, using the XelPleX system. XelPleX™ is the new generation of label-free interaction analysis platform.
The benzenesulfonamide is an inhibitor of the human Carbonic Anhydrase enzyme type II (CAII). The CAII catalyzes the hydration of carbon dioxide. Its role is to maintain acid-base balance in blood and tissues. Defects in this enzyme are associated with osteopetrosis and renal tubular acidosis. Renal CAII allows the reabsorption of sodium ions in the proximal tubule.





Benzenesulfonamide

Figure 1: Studied model

Materials and Method

CAII immobilization using SPRi-CFM on a CMD200MD SPRi-Biochip™

The CMD200MD SPRi-Biochip™ is a hydrogel made of carboxymethyldextran. The CMD200MD SPRi-Biochip™ is characterized by a thickness of 200 nm and a medium density. The CMD-200MD SPRi-Biochip™ is activated using an EDC/NHS solution in preparation for amine coupling.
CAII prepared in 10 mM sodium acetate at pH 5.0 and at a concentration of 2 µM was immobilized on the SPRi-Biochip™ activated surface using the SPRi-Continuous Flow Microspotter (SPRi-CFM).

The SPRi-CFM uses continuous flow deposition to immobilize up to 48 molecules in a single printing run. Three printing runs can be performed on a single biochip (up to 144 spots per chip can be generated). The microfluidic immobilization improves the spot homogeneity and gives a higher immobilization level. For this experiment, the flow rate of the SPRi-CFM was set to 15 µL/min and the contact time to 15 minutes.
A reference protein was also immobilized in the same conditions for referencing purposes. Each protein was immobilized in six replicates. After the immobilization procedure, the SPRi-Biochip™ was blocked using 1M ethanolamine.

Blue spots correspond to CAII
Pink spots correspond to reference spots.

Figure 2: Image of the printed SPRi-Biochip™.

SPRi Experimental Details

The printed SPRi-Biochip™ was then inserted into the XelPleX™ system. The running buffer was 10 mM PBS pH 7.4 3 % DMSO and the working temperature was set to 25°C.
Then, 400 µL of benzenesulfonamide was injected at a flow rate of 100 µL/min. The benzenesulfonamide was injected at five increasing concentrations following a three-fold dilution series: 1, 4, 11, 33 and 111 µM.

Results and Discussion

Figure 3 shows reference-subtracted kinetic curves obtained after injections of benzenesulfonamide at 1, 4, 11, 33 and 111 µM.

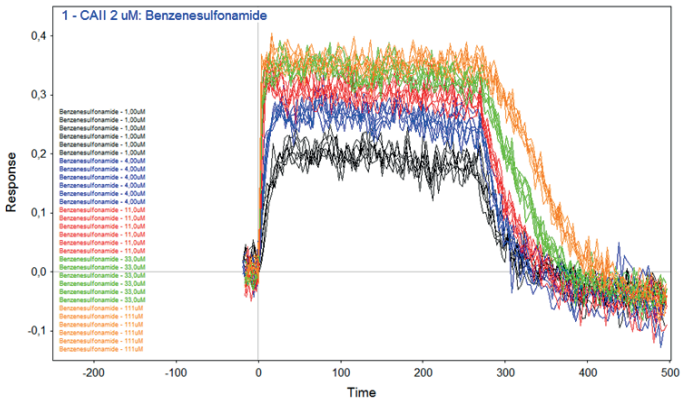


Figure 3: Reference-subtracted kinetic curves overlay after injections of benzenesulfonamide molecule at 1, 4, 11, 33 and 111 µM.

Monitoring the binding of benzenesulfonamide to CAII is observed for all the concentrations injected. Saturation level of CAII spots is reached for the highest concentration injected (111 µM). Association and dissociation of the benzenesulfonamide-CAII complex are very fast. For each concentration injected, a plateau is observed which corresponds to the equilibrium state of the complex.

Affinity between CAII and benzenesulfonamide was determined at the equilibrium state using the EzFit software (Figure 4).
This software is suitable for processing multiplexed data intuitively. The SPRi signal obtained on reference spots were used for referencing.

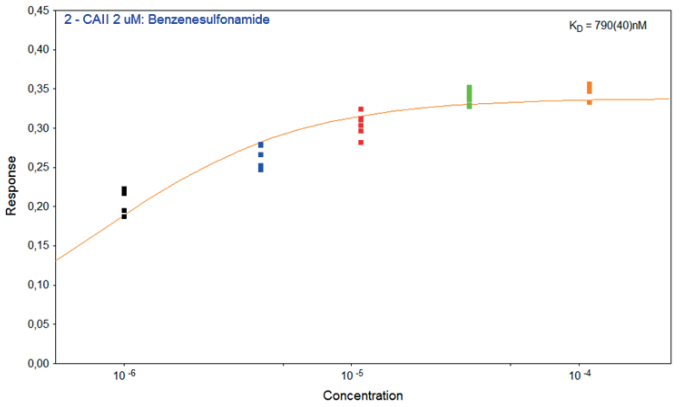


Figure 4: Equilibrium analysis of CAII – benzenesulfonamide interactions

The affinity calculated at the equilibrium between CAII and benzenesulfonamide is 0.8 µM which is correlated with the bibliography (Papalia et al., 2006[1]).

[1] Papalia, G.A., Leavitt, S., Bynum, M.A., Katsamba, P.S.,Wilton, R.,Qiu, H., Wang, S., Bindu, L., Phogat, S., Giannetti, A.M., Ryan, T.E., Pudlak, V.A., Matusiewicz, K., Michelson, K.M., Nowakowski, A., Pham-Baginski, A., Brooks, J., Tieman, B.C., Bruce, B.D., Vaughn, M., Balsh, M., Cho, Y.H., De Wit, M., Smets, A., Vandersmissen, J., Michiels, L. And Myszka, D.G., 2006, Comparative analysis of 10 small molecules binding to carbonic anhydrase II by different investigators using Biacore technology, Analytical Biochemistry 359, 94-105.

Conclusion

This application note validates the limit of detection in term of lowest molecular weight molecules for the XelPleX system. Indeed, the binding of a 157 Daltons molecule was monitored using the XelPleX sytem. Not only was the detection of a 157 Daltons molecule possible but also the affinity of the molecule to the CAII enzyme was determined by injecting increasing concentrations of benzenesulfonamide. The combination of the XelPleX instrument with the SPRi-CFM (a continuous flow printing system) on 3D surface chemistries was needed to reach the detection of small molecules as low as 157 Daltons of molecular weight.
Detection of low molecular weight molecules combined to the large working area of the SPRi-Biochip™ and the multiplexing capabilities of SPRi systems offers a great potential for pharmaceutical and agri-food industries.

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