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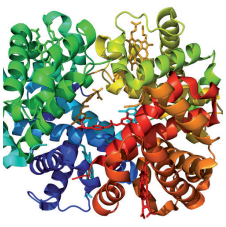
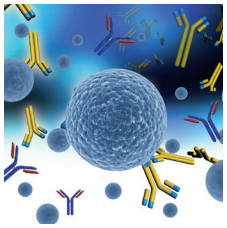
Surface Plasmon Resonance imaging

The Detection of a Low Molecular Weight Enzyme Inhibitor using the OpenPlex™ system





Application Note
Biomedical
SPRi AN39

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Introduction

The use of low molecular weight molecules in the industries of drug discovery and agri-food has become increasingly important over the course of a century of drug research and science development in agriculture and in pharmaceutical field.

Drug discovery is the combination of two sciences: chemistry and pharmacology. It began when chemists became masters of their science and applied it to other sciences, and when pharmacology became recognized as a scientific discipline in its own right[1].

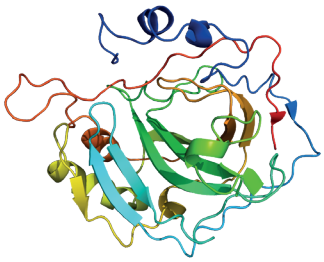
In drug discovery, low molecular weight molecules (or small molecules) are of particular interest because a majority of them are easy to characterize, non-immunogenic, stable and they have good potential of tissue penetration.

On the agri-food side, science became involved in agriculture with the research work of the German agronomist, Justus von Liebig. In 1840, his theory on the mineral nutrition of plants was a revolution in the field of agriculture, as it leads to the emergence of fertilizers[2].

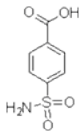
One example of an application on low molecular weight molecules, in the agri-food industry, is to detect traces of food contaminants like some toxins, particularly now, since new legal norms are regularly updated. Thus, the need for tools to study the detection and the affinity of such small molecules increases daily. Sensitive biosensors like Surface Plasmon Resonance (SPR) can play this role.

This application note shows how we can detect low molecular weight molecules with the OpenPlex™, the affordable and versatile SPRi platform.

We monitored the interaction between the 4-sulfamoyl benzoic acid (CBS), a 201 Dalton molecule, and the carbonic anhydrase enzyme type II (CAII) (**Figure 1**) with SPRi.
CBS is an inhibitor of the human CAII. The CAII catalyzes the hydration of carbon dioxide. Its role is to maintain an acid-based balance in blood and tissues. Defects in this enzyme are associated with osteoporosis and renal tubular acidosis. Renal CAII allows the reabsorption of sodium ions in the proximal tubule.



Carbonic Anhydrase II



CBS

Figure 1: Studied model

Materials and methods

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

The CMD200MD SPRi-Biochip™ is a hydrogel made of carboxymethyldextran. The SPRi-Biochip™ CMD 200MD is characterized by a thickness of 200 nm and a medium density. It is activated using an EDC/NHS solution in preparation for amine coupling.
CAII prepared in 10mM sodium acetate at pH 5.0 and at a concentration of 2µM 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 (and 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 15µL/min and the contact time to 15 minutes.
Two reference proteins were also immobilized under the same conditions for referencing purposes. Each protein was immobilized in multiple replicates (10 spots for reference proteins and 16 spots for CAII, **Figure 2**). After the immobilization procedure, the SPRi-Biochip™ was blocked using 1M ethanolamine.

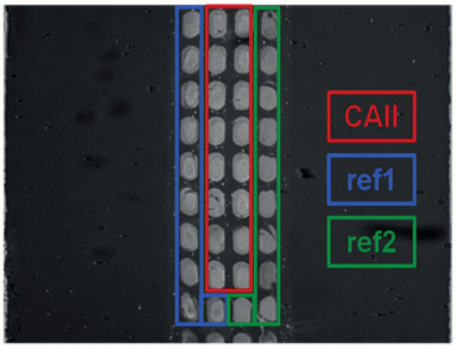


Figure 2: Image of the printed SPRi-Biochip™. CAII spots are framed in red, reference protein 1 is framed in blue and reference protein 2 is framed in green.

SPRi experimental details

The printed SPRi-Biochip™ was then inserted into the OpenPlex™ system. The running buffer was 10mM PBS pH 7.4 3% DMSO.
Then, 400 µL of CBS was injected at a flow rate of 100µL/min. The CBS was injected at four increasing concentrations, following a three-fold dilution series: 1, 4, 11 and 33µM.

Results and discussion

Figure 3 shows averaged and reference (reference 1)-subtracted kinetic curves obtained after injections of CBS at 1, 4, 11 and 33µM.

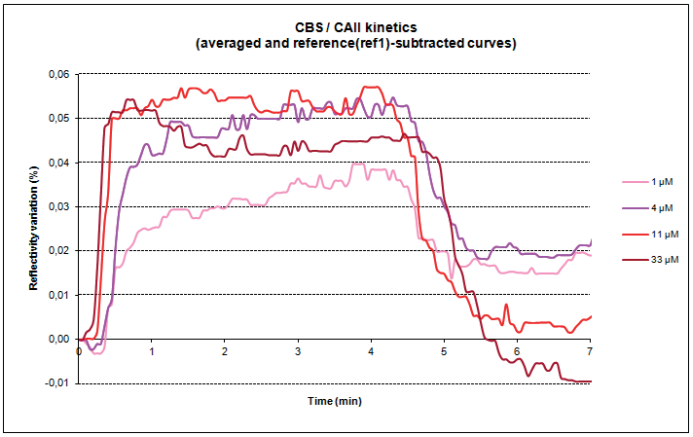


Figure 3: Averaged and reference (reference 1)-subtracted kinetic curves overlay after injections of CBS molecule at 1, 4, 11 and 33µM.

Monitoring the binding of CBS to CAII is observed for all the concentrations injected. Saturation level of CAII spots is reached for the highest concentration injected (33µM). Association and dissociation of the CBS-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 CBS was determined at the equilibrium state using the ScrubberGen software (**Figure 4**).
This software is suitable for processing multiplexed data intuitively. The SPRi signal obtained on reference 1 spots was used for referencing.

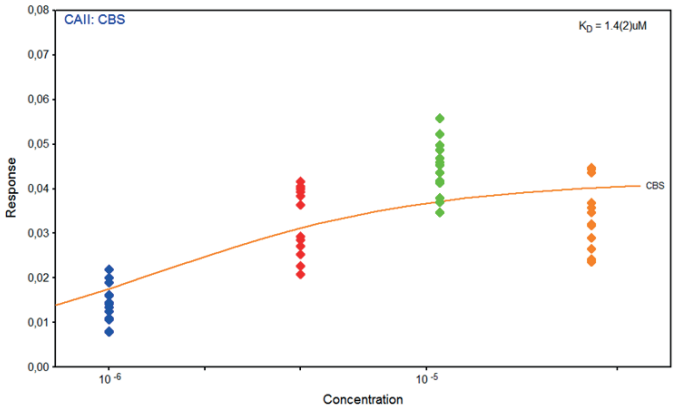


Figure 4: Equilibrium analysis of CAII – CBS interactions

The affinity evaluated at the equilibrium between CAII and CBS is 1.4µM, which is correlated with the bibliography[3].

Conclusion

This application note demonstrates the feasibility of small molecules detection and their affinity study using the OpenPlex™ system. Through the CBS-CAII model, we are able to validate the detection of a 201 Daltons molecule. Moreover, we determined the affinity of the studied model by injecting increasing concentrations of CBS on the SPRi-Biochip™ functionalized with 3D chemistry. Combining this platform with the printing using the SPRi-CFM (a continuous flow printing system) enabled us to detect this small molecule.
This application note demonstrates how the OpenPlex™ Platform is useful to new fields like drug discovery and agri-food research, which join the vast number of applications for the OpenPleX™.

References

[1] Drews J. and al., 2000, Drug Discovery: A Historical Perspective, Science 287, 1960-1964.

[2] Von Liebig J.F. and Playfair L.P.B., 1843, Chemistry in its application to agriculture and physiology.

[3] Papalia G.A. and al, 2006, Analytical Biochemistry 359, 94-105.

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