

Small molecule detection using Surface Plasmon Resonance imaging

Surface Plasmon Resonance imaging (SPRi) enables a large number of different molecular interactions to be monitored simultaneously and in parallel in a single experiment. Throughput is generally superior to other channel-based SPR systems and detection sensitivity is relatively high allowing fast matrix experiments and analyses to be undertaken. This application note shows that in addition to the benefits of speed and throughput, the unique technology used within the HORIBA SPRi instruments is also suitable for the detection and study of small molecules as low as 201 Da.

For this study, a protein was immobilized on a SPRi-Biochip™ covered with a 3D surface chemistry (Dextran) and the interaction with a small molecule was monitored in real time by SPRi providing a clear illustration of the small molecule capabilities of the technique.

Materials and methods

SPRi-CFM printing of the SPRi-Biochip™

The SPRi-Biochip™ covered with a Dextran surface was activated using an EDC/NHS solution during 10 minutes in preparation for amine coupling.

Figure 1 shows the checkerboard pattern used for immobilizing molecules in an array format (spots). Carbonic Anhydrase isozyme II (CAII) prepared in 10 mM sodium acetate was immobilized in a 2-fold dilution series format to the activated surface of the SPRi-Biochip™ using the SPRi-Continuous Flow Microspotter (SPRi-CFM). The SPRi-CFM uses flow printing and a back-and-forth movement to immobilize the molecules. This allows for a better spot homogeneity and a better control over the deposition process. For this experiment, the flow rate was set to 60 µL/min and the contact time to 20 minutes. The highest spotting concentration was 50 µg/ml and the lowest 6 µg/ml. A reference molecule was also immobilized at the same concentrations for referencing purposes. Spots were immobilized in quadruplicate. After the immobilization procedure, the SPRi-Biochip™ was blocked using 1 M ethanolamine.

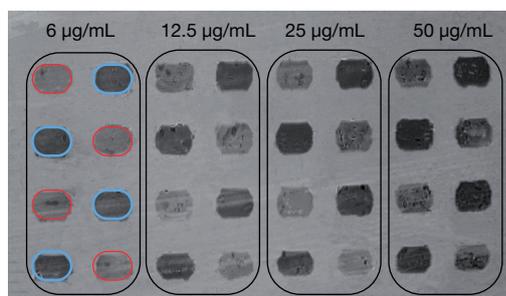


Figure 1: Image of the SPRi-Biochip™. Blue spots correspond to CAII and red spots correspond to reference spots.

SPRi experimental details

The spotted SPRi-Biochip™ was then inserted into the SPRi-PlexII™ system from HORIBA Scientific - GenOptics. The running buffer was 1 X PBS (+ 0.1 mg/mL BSA + 0.005% p20) and the working temperature set to 25°C. Then, 900 µL of 50 µM 4-carboxybenzenesulfonamide (CBS) was injected into the fluidic system at 300 µL/min. The molecular weight of CBS is 201 Da and was selected due to its relatively small molecular weight. The binding of CBS to CAII was monitored in real time. Figure 2 shows the 3D rendering of the interaction between the two molecules.

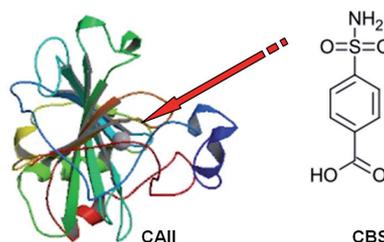


Figure 2: Interactions between CAII and CBS

Results and discussion

Figure 3 shows the sensorgrams (Kinetic binding curves) obtained for the binding of 50 μM CBS to CAII spots and reference spots. Note that since the responses from all the reference control spots are similar, it may be possible to use far fewer reference spots in any future experiments and maximise the total number of active CAII spots that can be used.

The interaction between CBS and CAII is specific whatever the immobilization concentration of CAII on the SPRI-Biochip™ surface.

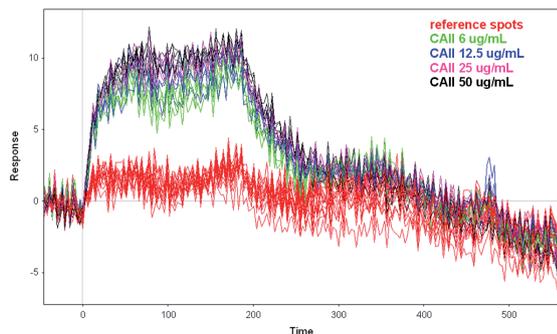


Figure 3: Real time sensorgrams obtained after the injection of 50 μM CBS.

Figure 4 shows the responses obtained after subtraction of the average reference response. It is thus possible to extract quantitative information regarding the amount of CBS that bound to CAII spots (Figure 5). The best interaction signal was obtained for CAII immobilized at 50 $\mu\text{g/mL}$.

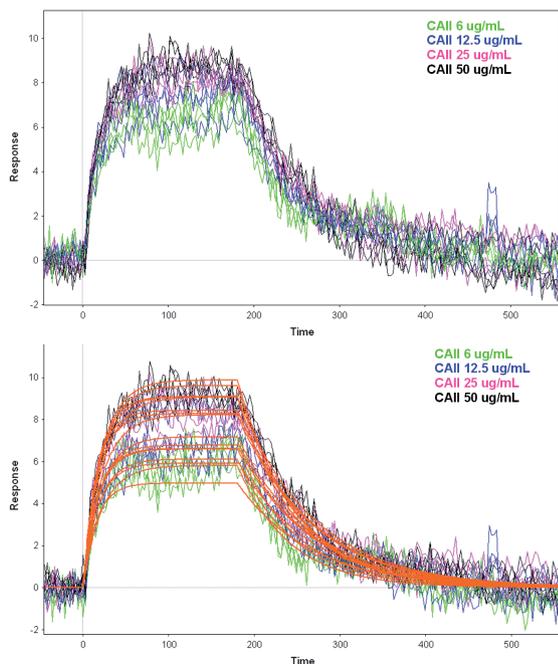


Figure 4: (top) Reference-subtracted sensorgrams obtained after the injection of 50 μM CBS.
(bottom) Individual fit using a 1:1 interaction model

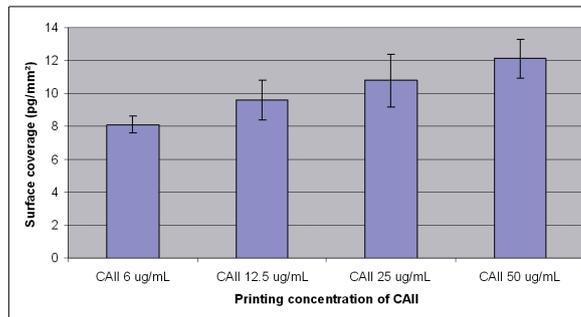


Figure 5: Amount of CBS bound to CAII for different immobilization concentrations

Conclusion

This application note shows that it is possible to detect small molecules as low as 201 Da using the HORIBA Scientific-GenOptics technology. The combination of flow printing on 3D surface chemistries using the SPRI-Continuous Flow Microspotter (SPRI-CFM) improves significantly the immobilization procedure, hence the detection sensitivity of SPRI still further. The matrix (grid) arrangement on the biochip enables the protein (probe) to be immobilised with different conditions and thence to work in a multiplexed high-throughput format.

The ability to detect small molecules and the multiplexed biochip capability shows great promise for the development of applications in high-throughput drug screening where small molecule sizes are involved- saving time and consumable costs for the operator.