HORIBA Scientific

Technical Note

SPRi

SPR-TN02

Multiplexed Detection of Antigens Using an Antibody Biochip

HORIBA Scientific unique Surface Plasmon Resonance imaging (SPRi) instruments enable a large number of different label-free molecular interactions to be monitored simultaneously and in parallel in a single experiment. Throughput presents superior capacity in comparison with channel-based SPR systems, allowing fast matrix experiments and analyses to be undertaken.

In this technical note, the multiplexing capability of SPRi will be demonstrated using an antibody SPRi-Biochip™. The biochip contains several antibodies either useful for food (milk, egg white) and pollen (birch) allergy detection, directed against proteins from biological fluids (serum, saliva) or bought from different suppliers for comparison.

Material and Method

Antibody immobilization using SPRi-CFM on a CS SPRi-Biochip™

The CS SPRi-Biochip™ is made of a self-assembled monolayer of polyoxyde ethylene glycol activated using an EDC/sulfo-NHS solution in preparation for amine coupling. The different antibodies immobilized on the biochip are shown in Table 1.
Figure 1 shows the pattern used for immobilizing molecules in an array format (spots). All antibodies were prepared at a final concentration of 0.7 µM (100 µg/mL) in 10 mM sodium acetate at pH 4.5, and immobilized to the activated surface of the SPRi-Biochip™ using the SPRi-CFM. The SPRi-CFM uses 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). Immobilizing with flow generates increased spot homogeneity and higher immobilization levels. Each antibody was immobilized in four replicates. After the immobilization procedure, the SPRi-Biochip™ was blocked using 1 M ethanolamine and saturated using 1 % BSA.

Antibodies immobilized on the biochip	Supplier
Polyclonal anti-ovalbumin	TEBU
Polyclonal anti-ovalbumin	TETRACORE
Polyclonal anti-streptavidin	TEBU
Polyclonal anti-streptavidin	SIGMA
Polyclonal anti-lectin	SIGMA
Polyclonal anti-transferrin	TEBU
Polyclonal anti-α amylase	SIGMA
Polyclonal anti-α lactalbumin	EUROMEDEX
Monoclonal anti-Bet v 1	STALLERGENES
Polyclonal mouse IgG (negative control)	SIGMA

Table 1: List of antibodies immobilized on the SPRi-Biochip™

Figure 1: Image of the printed SPRi-Biochip™ and spotting matrix

SPRi experimental details

The printed SPRi-Biochip™ was then inserted into the SPRi-PlexII™ system. The running buffer was 10 mM PBS pH 7.4 and the working temperature was set to 25° C.
Then, 200 µL of each corresponding antigen were injected into the fluidic system at a flow rate of 50 µL/min. The surface was regenerated using 0.1 M glycine-HCl pH 2.0 or 10 mM NaOH after each antigen injection.

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Results and Discussion

Figure 2 shows the average kinetic curves obtained for the binding of each antigen to all antibody spots immobilized after subtraction of the negative control.

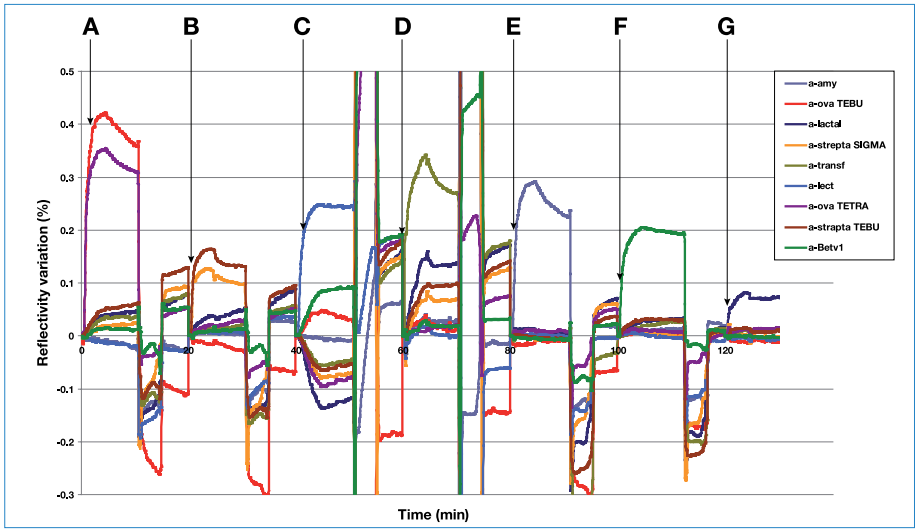


Figure 2: Average kinetic curves (4 spots) after subtraction of the negative control for the following antigen injections: A: 230 nM (10 µg/mL) ovalbumin, B: 170 nM (10 µg/mL) streptavidin, C: 95 nM (5 µg/mL) lectin, D: 6.3 µM (500 µg/mL) transferrin, E: 80 nM (5 µg/mL) α amylase, F: 57 nM (5 µg/mL) recombinant Bet v 1, G: 284 nM (10 µg/mL) α lactalbumin

Antibody screening

Working in an array format allows for the rapid comparison of different antibodies targeting the same antigen. To illustrate the antibodies comparison, here, two antibodies targeting ovalbumin and streptavidin (from two different providers) are immobilized on the same biochip at the same concentration. Only one injection of the antigen permits to compare each antibody response. The anti-ovalbumin and anti-streptavidin antibodies from TEBU give the highest SPR response (about 40 % more signal). They are also highly specific as the signal for the other antibodies is negligible on the kinetic curves and on the SPR difference images (Figure 3). White spots on the SPR difference image correspond to areas where binding has occurred.

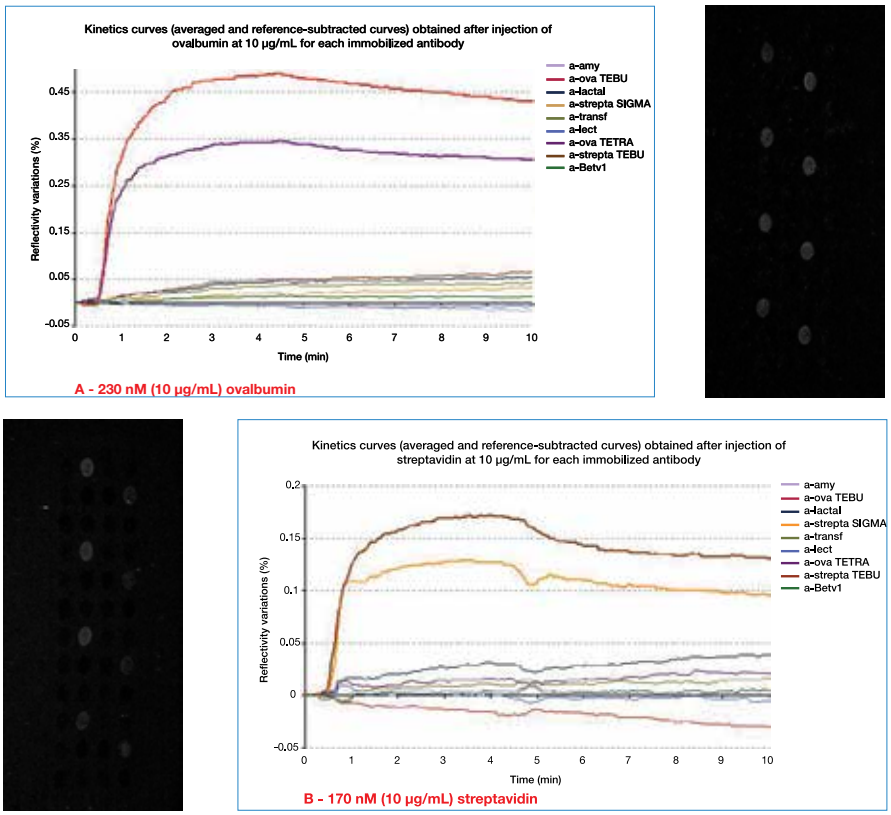
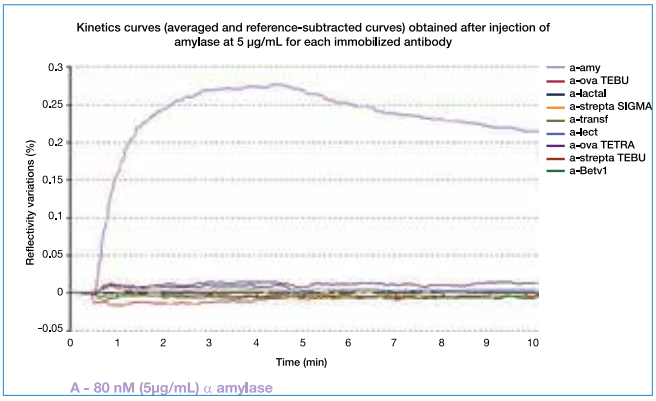
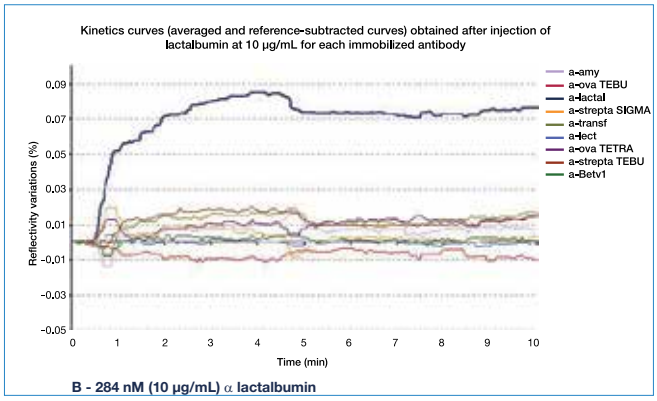


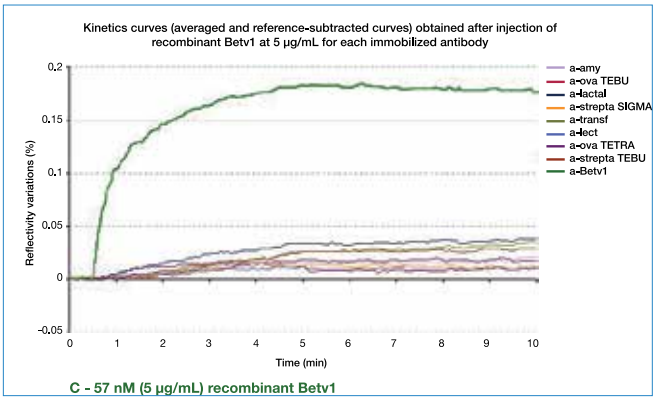
Figure 3: Kinetic curves and difference images obtained after the injection of A/ovalbumin and B/ streptavidin at 10 µg/mL.

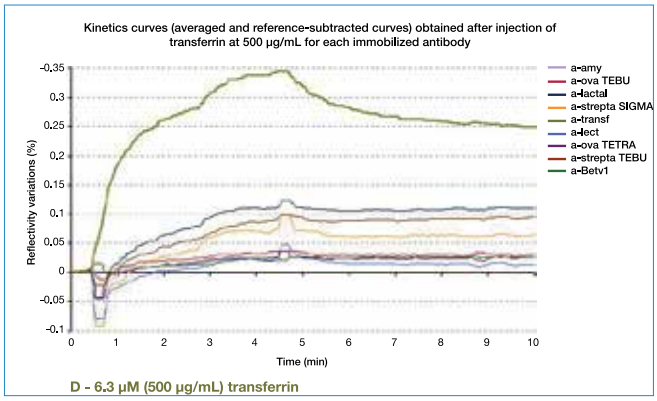
Antibody specificity

The specificity of the antigen-antibody interaction can also be easily determined using the multiplex approach. For example, anti-α amylase, anti-α lactalbumin and anti-Bet v 1 are extremely specific for their antigens (signal of all other antibodies remains to baseline) compared to anti-transferrin and anti-lectin (Figure 4). The negative signal obtained after injection of lectin is due to the high response of the negative control spot (mIgG).









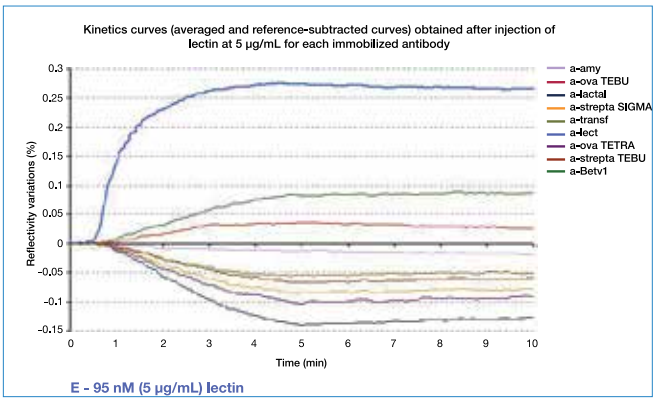


Figure 4: Kinetic curves obtained after the injection of A: α amylase, B: α lactalbumin, C: recombinant Bet v 1, D: transferrin and E: lectin.

Conclusion

This technological note shows the great potential of Surface Plasmon Resonance imaging for the screening of molecules. Working in an array format allows the direct comparison of the binding events occurring on the biochip. It is possible to characterize multiple binding partners in terms of specificity, reactivity, kinetics parameters and affinity in a single experiment, saving on consumable and reagents costs. We see promising applications of Surface Plasmon Resonance imaging in the fields of antibody screening, epitope mapping and aptamer selection.

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