SPRi-0708.6-v2

Application Note

Biochips™ Robustness of Pyrrolated-Conjugate Antibody/Antigen Interaction

Hundreds of successive interactions-regeneration steps within the process of measuring biological interactions by SPRi technology (Surface Plasmon Resonance imaging) is a demand we can provide. One of the surface chemistries combined with an electrochemical process developed by Genoptics consists on the immobilization of pyrrole-conjugate biomolecules on SPRi Biochip™ with polypyrrole copolymerisation. Using this technique for receptors immobilization on sensor chip, we show that more than one hundred regenerations are tolerated without loss of activity. The biological interaction chosen to illustrate this demand is the anti-ovalbumin / ovalbumin model.

Materials and methods

Preparation and immobilization of pyrrole-anti-ovalbumin and pyrrole-mouse IgG conjugates

Anti-ovalbumin is conjugated with pyrrole-NHS in phosphate buffer saline for two hours at room temperature. At the end of process, anti-ovalbumin is desalted in phosphate buffer containing 50 mM PO_4 buffer, 50 mM NaCl, 10% glycerol.
The negative control (mouse IgG) is conjugated with pyrrole-NHS in the same way as anti-ovalbumin.

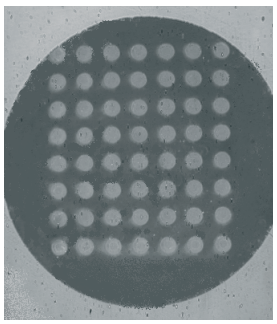

Figure 1: Image of the Biochip™

The spotting solutions (phosphate buffer with 10% glycerol) contain 20 mM free pyrrole and one of the antibody at 8 µM concentration. The grafting of the antibodies on the biochip is carried out with a SPRi Arrayer™ by an electrochemical process. For polymerization of pyrrole-antibodies copolymer, an electrical pulse (2V for 100ms) was generated between the working electrode (prism gold surface) and the counter electrode (located in the arrayer pin). The tip was rinsed with distilled water after each spotting.
Forty nine anti-ovalbumin and seven mouse IgG spots were grafted on the SPRi Biochip™.

SPRi experiment

After spotting, the biochip is introduced into the SPRi Plex™ instrument. The running buffer is 10mM PBS.

Injected solutions

Ovalbumin diluted at 20 µg/mL in the running buffer (10 mM PBS) and glycine 100mM / HCl pH=2.0 were alternatively injected (up to 112 times) on the flow cell and interaction/regeneration steps can be monitored in real time without labeling.

The injections were carried out automatically.

Results and discussion

SPRi quantification of proteins binding on the biochip

Interaction curves

For readability purpose, only four interaction / regeneration steps were shown below (figure 2).

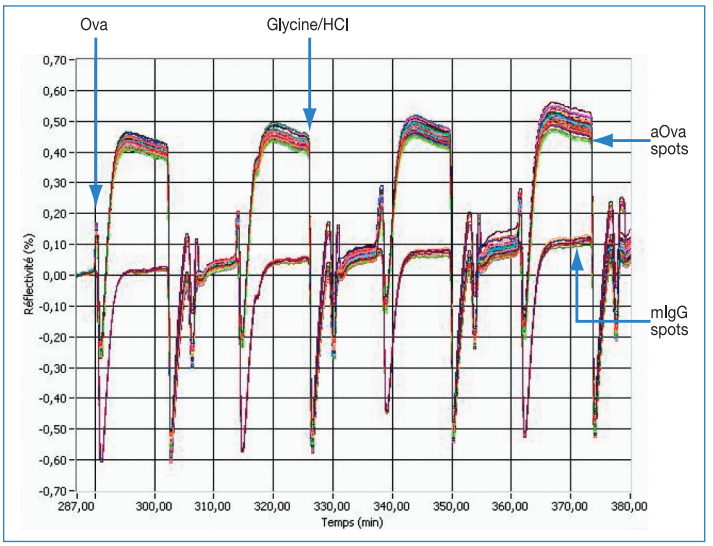

Figure 2: Interaction curves (interaction / regeneration) on anti-ovalbumin (aOva) spots and negative control spots (mouse IgG (mIgG)).

Specific interactions were observed between ovalbumin and its complementary antibody whereas only background was detected on the mouse IgG spots.

The amount of ovalbumin bound onto anti-ovalbumin spots was constant. These data indicated an out-and-out regeneration because the signal returned to the previous baseline. The slight drift can easily be corrected in subtracting values of negative control to those of anti-ovalbumin (figure 3).

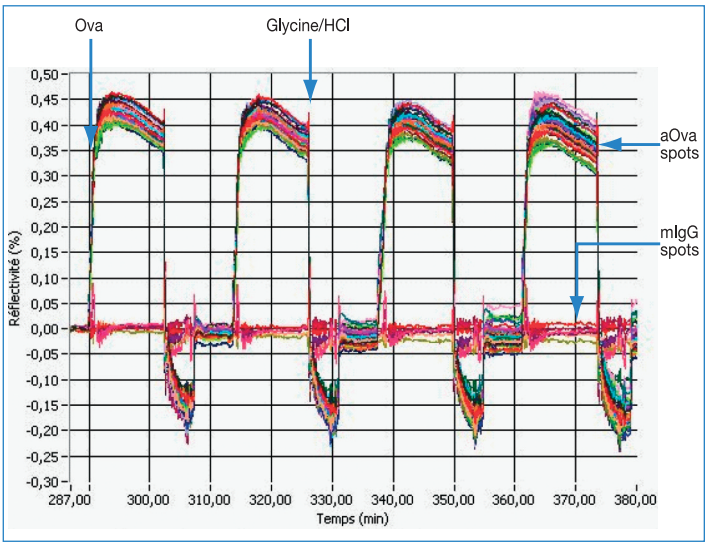

Figure 3: Kinetics curves after subtraction of the negative control

Proteins amount versus the injections number

Histogram below (figure 4) represents ovalbumin amount bound onto anti-ovalbumin antibodies. Each injection of ovalbumin was regenerated with glycine/HCl.

We can notice (Figure 4) that the amount of proteins, interacting with anti-ovalbumin antibodies, decreases at the beginning of the experiment. After the 25th injection, the signal remains stable. Indeed, this is due to a stabilization phase: at the beginning, some anti-ovalbumin molecules are not totally grafted on the biochip surface and are removed when the biochip is regenerated.

Conclusion

We demonstrate here the robustness of our system. We performed 112 ovalbumin injections each followed by injection of regeneration buffer (Glycine/HCl). After more than one hundred regenerations, the biochip remains active as indicated on histogram (figure 4).

Grafted pyrrolated antibody on SPRi Biochip™ tolerates more than one hundred protein-interactions / regenerations without loss of activity.

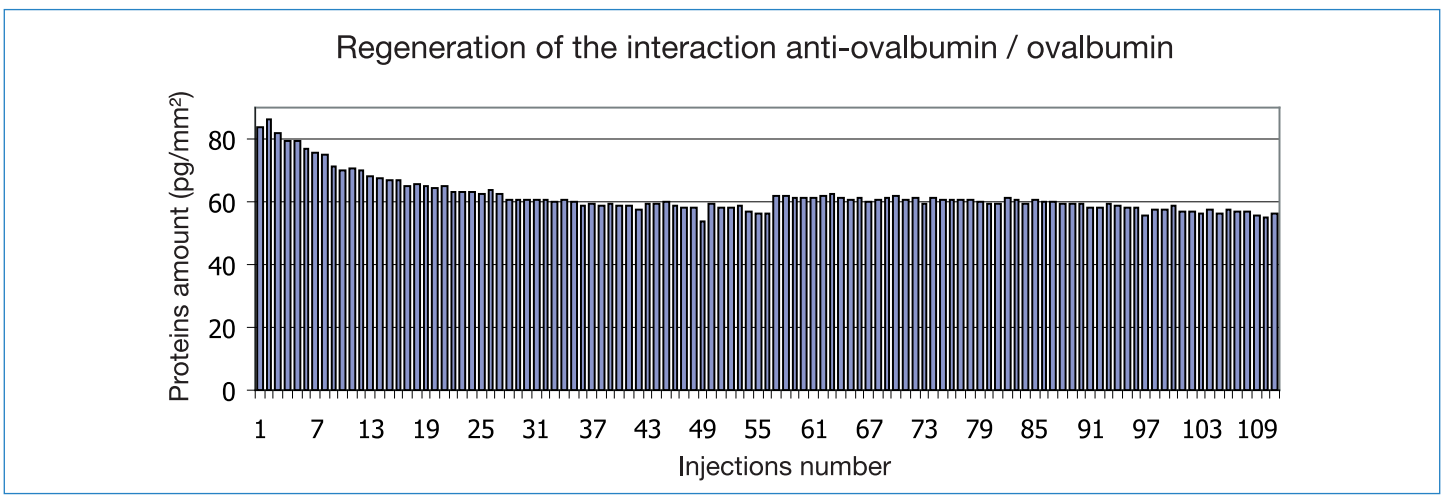

Figure 4: Proteins amount immobilized after injection of 20 µg/mL ovalbumin versus the number of regenerations.

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GENOPTICS BIO INTERACTIONS

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