SPRi-0809.10-v1.1

Application Note

Antibody/Protein interaction on a biochip functionalized with a Cystamine/Glutaraldehyde layer, biochip robustness

The increased demand for hundreds of successive interaction-regeneration steps within the process of measuring biological interactions by SPRi technology (Surface Plasmon Resonance imaging) has led Genoptics to develop new surface chemistries. One of the surface chemistries developed consists of the formation of a cystamine/glutaraldehyde layer on the biochip surface on which receptors can be directly spotted before introduction of the chip into the SPRi system. Using this surface chemistry for receptor immobilization we are able to show that more than one hundred regenerations are tolerated without loss of activity. The biological interaction chosen to illustrate this is the anti-ovalbumin / ovalbumin model.

Materials and methods

Preparation of the Cystamine/Glutaraldehyde layers

Firstly, the biochip surface was cleaned by UV-Ozone for one hour. Immediately after, the biochips were quickly immersed in a 25 mM cystamine/ 90% ethanol solution for 2 hours with agitation. The biochip was washed for 5 minutes with a 90% ethanol solution, dried, then rinsed for 5 minutes with a 10 mM PBS solution and dried again. Finally, the biochip was dipped into a 2.5% glutaraldehyde/ 10 mM PBS solution for one hour with agitation, then rinsed with a PBS solution and dried.

Immobilization of antibodies

The immobilization step was performed by an SPRi Arrayer™ directly on the functionalized biochip surface before introducing the chip into the SPRi system.

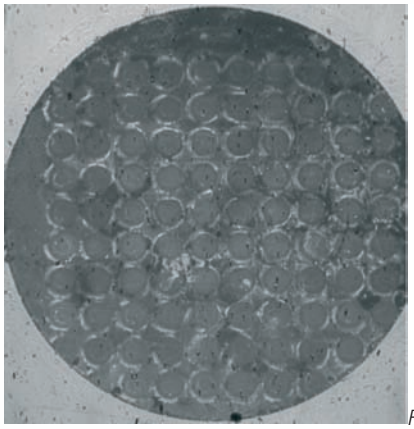

Figure 1: Image of the biochip

The spotting solution (10mM PBS solution with 10% glycerol) contained 6.7 µM anti-ovalbumine antibodies. The negative control solution contained 6.7 µM mouse IgG and the tip was rinsed with distilled water after each spotting.

Seventy anti-ovalbumin and twenty three mouse IgG spots were grafted onto the biochip.

Injected solutions

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

SPRi experiment initialisation

The biochip surface without antibody immobilization was blocked by the injection of a 10 mM glycine/ 10 mM PBS solution and rinsed with 10 mM PBS. The non specific sites were saturated with injection of a 1% BSA solution.

Results and discussion

SPRi quantification of protein binding on the biochip

The injections were carried out automatically.

Specific interactions were observed between ovalbumin and its complementary antibody whereas only background levels were 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 as the signal returned to the previous baseline (figure 2).

Figure 3 represents the amount of ovalbumin on the anti-ovalbumin antibodies. Each injection of ovalbumin was regenerated with glycine/HCl.

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 in figure 3.
After 111 regenerations cycles, the interaction signal varied by only ± 4%.

Antibody-protein interactions on biochips functionalized with a Cystamine/Glutaraldehyde layer will tolerate more than one hundred regenerations without loss of activity.

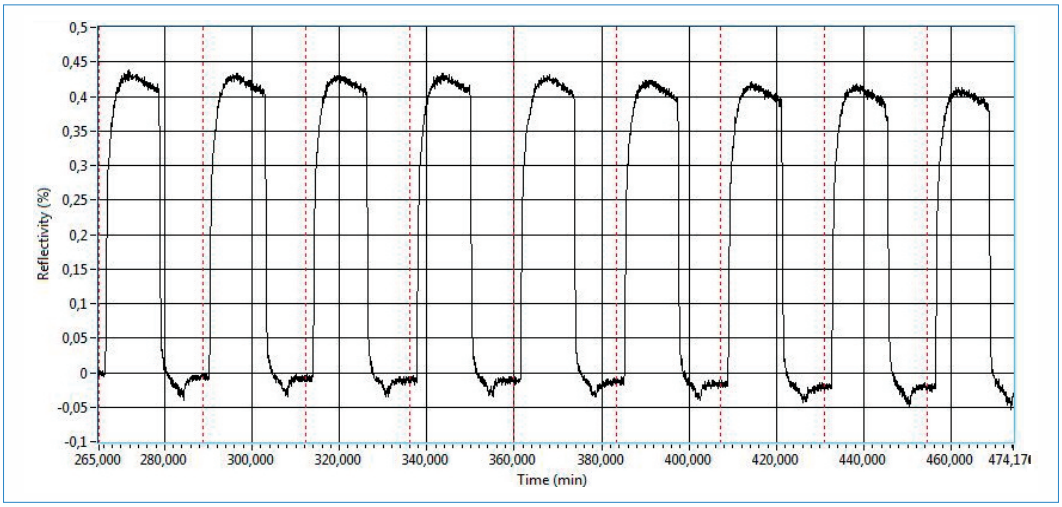

Figure 2: A series of antibody-antigen interaction curves after subtraction of the negative control

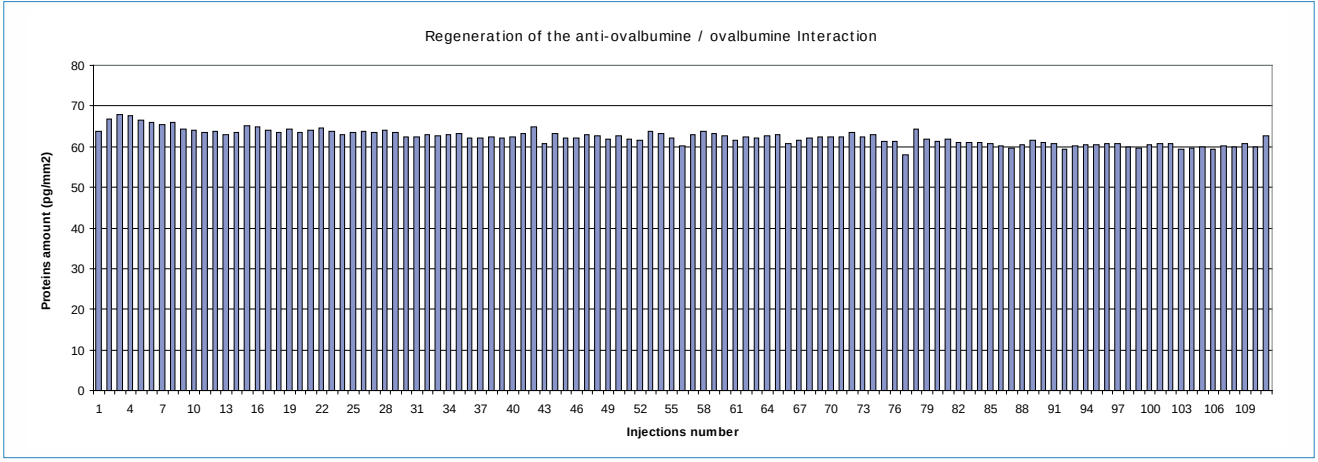

Figure 3: Protein amounts immobilized after injection of 20 µg/mL ovalbumin versus the number of regenerations.