SPRi-0809.9-v1.1

Application Note

Reproducibility of Antibody / Protein Interactions on Cystamine / Glutaraldehyde Functionalized Biochip

Good reproducibility within the process of measuring biological interactions by SPRi technology (Surface Plasmon Resonance imaging) is a demand Genoptics can meet. One of the surface chemistries developed by Genoptics 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 show an intra-biochip with acceptable reproducibility
The biological interaction chosen to illustrate this demand is the anti-ovalbumin / ovalbumin model.

Materials and methods

Preparation of the Cystamine/Glutaraldehyde layers

Firstly, the biochip surface was cleaned by UV-Ozone treatment and then immediately immersed in a 25 mM cystamine / 90% ethanol solution for 2 hours with agitation. The biochip was then washed with a 90% ethanol solution and dried, then rinsed with a 10 mM PBS solution. 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.

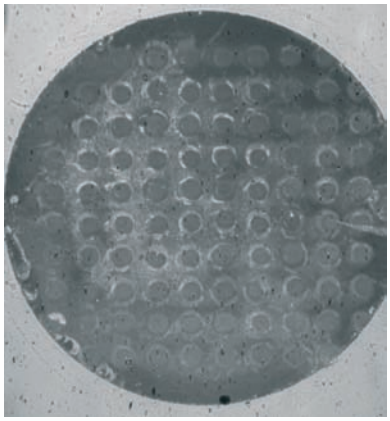

Figure 1: Image of the biochip

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

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

Injected solutions

200 µL of ovalbumin solutions were injected, between 1 µg/mL and 100 µg/mL concentration diluted in the running buffer (10 mM PBS).

100 mM Glycine/HCl (pH=2.0) solution was used to regenerate the interactions.

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. Then the non specific sites were saturated with an injection of a 1% BSA solution.

Results and discussion

Two kinds of experiments were carried out on the same biochip to compare the intra biochip response.

- The first experiment consisted of successive injections of ovalbumin solutions with increasing concentration without regeneration.

The signal obtained on the negative control spots was subtracted from the positive signal.

	Ovalbumin concentration (µg/mL)		
	1	10	100
Reflectivity variation (%)	0.12	0.21	0.25
Stdev	0.02	0.03	0.03

- During the second experiment, ovalbumin solutions of increasing concentration were injected. However, the anti-ovalbumin/ovalbumin interactions were regenerated by injection of a glycine/HCl solution between protein injections. The signal obtained on the negative control spots was subtracted from the positive signal.

	Ovalbumin concentration (µg/mL		
	1	10	100
Reflectivity variation (%)	0.10	0.25	0.30
Stdev	0.01	0.02	0.03

Specific interactions were observed between ovalbumin and its complementary antibody whereas only background levels were detected on mouse IgG spots irrespective of whichever method was used.

Conclusion

Biochips functionalized with Cystamine/Glutaraldehyde layers allow a good reproducibility between the anti-ovalbumin spots within the same chip. Moreover, this surface chemistry developed by Genoptics is an easy way to immobilize receptors on a biochip surface. No modification of the immobilized biomolecule is needed and we have shown that a hundred successive interaction/regeneration processes can be tolerated without loss of activity. (See Application Note SPRi-0708.6-v2).

Figure 2 compares the signal obtained on a biochip after successive injections of ovalbumin of increasing concentration, with and without regeneration between each injection.

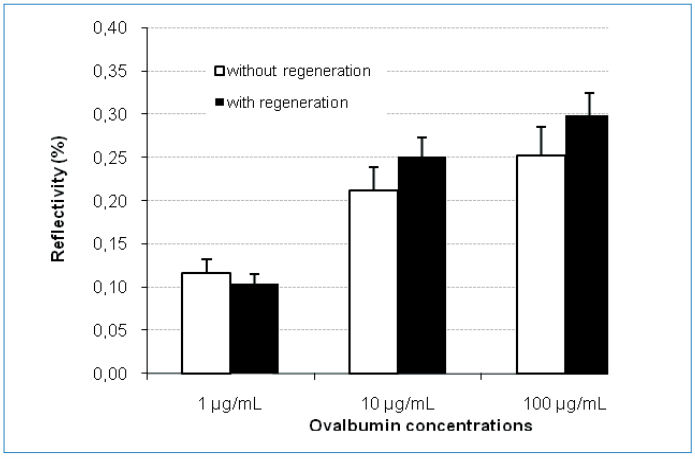

Figure 2: Signal (reflectivity %) after injections of ovalbumin solutions with increasing concentration

We observed a significant signal from a 1 µg/mL ovalbumin concentration.
The reproducibility between the spots from the same chips was good with roughly a 10% difference within the biochip.

Thus, the responses were similar, with and without regeneration whatever the injected concentration.