Biochip robustness of diazonium-aniline derivatives proteins

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 and immobilization of anti-ovalbumin and mouse-IgG aniline derivatives on the SPRi-Biochip™

Anti-ovalbumin is first functionalized with 4-carboxymethylaniline (CMA) in a carbonate-bicarbonate buffer for 2 hours at room temperature. At the end of the process, anti-ovalbumin is desalted and concentrated on column.
The negative control (mouse IgG) is conjugated with CMA in the same way as anti-ovalbumin.

The diazotation reaction of the aniline derivative leads to the formation of an aryl diazonium which generates an aryl radical following electrochemical reduction. The aryl radical attacks the gold surface and thereby the conjugate biomolecule is grafted on the biochip surface.
Before spotting, solution containing HCl and $NaNO_2$ is added to 8µM CMA-IgG and incubated for 10min. The grafting of the IgG on the biochip is carried out with an SPRi Arrayer™ using a reduction electrochemical process and an electrical pulse (-1V for 1000ms) which is applied between the working electrode (prism gold surface) and the counter electrode (located in the arrayer pin).

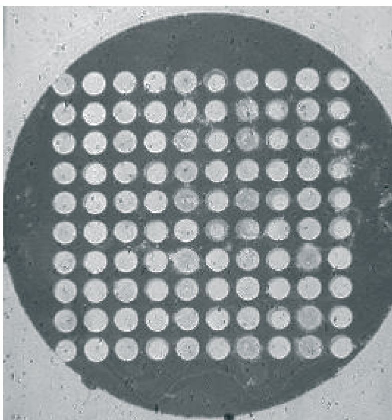

Figure 1: Image of the spotted SPRi-Biochip™

SPRi experiment

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

Injected solutions

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

On the biochip presented figure 1, fifty anti-ovalbumin and fifty mouse-IgG spots are grafted on the biochip.

Results and discussion

SPRi quantification of protein binding on the biochip

Interaction curves

The injections are carried out automatically. A part of the 112 injection steps is presented figure 2.

As the full kinetics curves for both families lack clarity (Figure 2), visualization of an average interaction curve of the interaction anti-ovalbumin / ovalbumine was regenerated automatically.

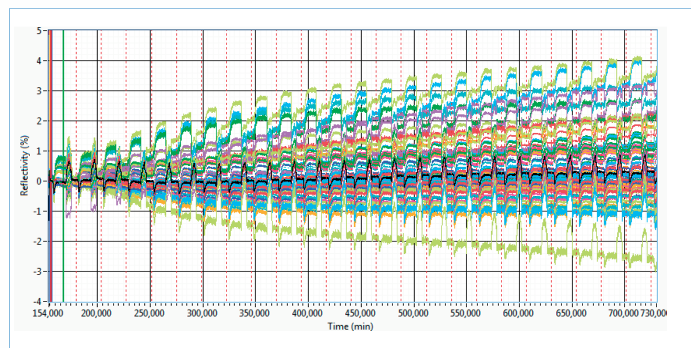

Figure 2: Kinetic curves (24 interaction / regeneration steps) on anti-ovalbumin (aOva) spots and negative control spots (mIgG).

Specific interactions are observed between ovalbumin and its complementary antibody whereas only background is detected on mouse IgG spots. So the negative control (mIgG) data are subtracted. The drift of the baseline can also be corrected

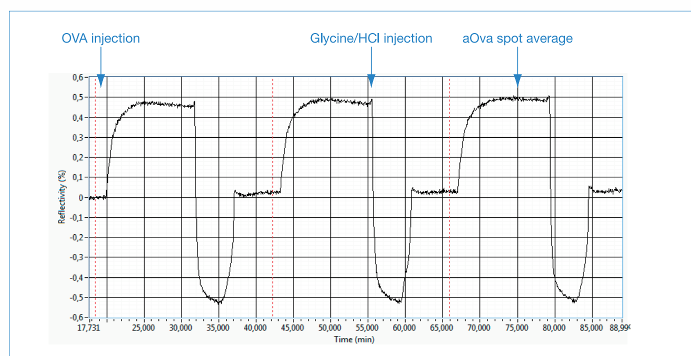

Figure 3: Average interaction curve (interaction / regeneration) on anti-ovalbumin (aOva) spots following negative control spots (mouse IgG) subtraction.

with the substraction of the negative control values to those of anti-ovalbumin. Results can be observed on figure 3 and for readability purpose, only three interaction /regeneration steps are shown.
The amount of ovalbumin binding onto anti-ovalbumin spots is constant. These data indicate an out-and-out regeneration because the signal returns to the previous baseline.

Protein amounts versus the injections number

Figure 4 represents the ovalbumin amount bound onto anti-ovalbumin antibodies. Each injection of ovalbumin was regenerated with glycine/HCl.

We can notice that the amount of proteins, interacting with anti-ovalbumin antibodies is stable. The first and the last injections after 111 regenerations are presenting the same amount of bound proteins.

Conclusion

We demonstrate here the robustness of our diazonium-aniline-protein derivatives Biochip™. 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 the histogram (figure 4).

Grafted diazonium-aniline conjugated antidody on SPRi-Biochip™ tolerates more than one hundred protein-interactions / regenerations without loss of activity.

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