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Application Note

Protein / GST-tagged Protein interaction on a multiplex biochip functionalized with a Cystamine / Glutaraldehyde / anti-GST layer

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The immobilization and the interaction studies of GST labelled proteins by SPRi technology (Surface Plasmon Resonance imaging) is a demand we can provide. One of the surface chemistries developed consists of an anti-GST functionalized biochip surface. Anti-GST antibody is grafted on a cystamine/glutaraldehyde layer on the biochip surface, so GST-receptors can be directly spotted before introduction of the chip into the SPRi system. Using this GST-derivative surface chemistry for partner GST-labelled probe immobilization we show specific and sensitive interaction of the system.The biological interaction chosen to illustrate this demand is the GST-calmodulin / calcineurin model.

Materials and methods

Preparation of the Cystamine/Glutaraldehyde layers

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

Immobilization of anti-GST

A 10µg/mL anti-GST antibody in 10mM PBS solution was coated on the whole chip surface for 24 hours. A 10mM glycine solution was finally dropped on the surface to react with the remaining free active surface group.

Spotting of GST-labeled proteins

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

The spotting solutions (10mM HBS and 10mM $CaCl_2$ solution with 10% glycerol) contained 10nM, 100mM or 1µM GST-calmodulin protein (GST-CaM1). The negative control solutions contained GST protein. Fifteen spots of each solution were directly dropped. The tip was rinsed with distilled water after each spotting.
Forty five GST-CaM1 and forty five GST spots (three concentrations of each) were grafted onto the bichip.

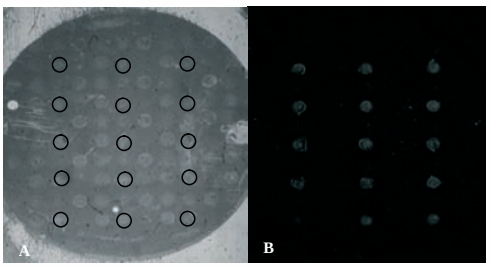

Figure 1: A: Image of the biochip with 1µM GST-CaM1 spots black indicated and B: SPR image of the biochip surface following injection of 500ng/mL calcineurin.

Injected solutions

Calcineurin solutions diluted from 10 to 500ng/mL in the running buffer (10mM HBS, 10mM CaCl2 pH 7.4) were injected. Regeneration step occurred with injection of 3mM EDTA in 10mM HBS solution onto the flow cell system following proteins interaction. All events were monitored in real time without labeling.

SPRi experiment initialization

After its functionalization, the biochip was introduced in the SPRi instrument. The flow rate was 50µL/min. The non specific sites were saturated with injection of a 1% BSA solution.

Results and discussion

SPRi quantification of protein binding on the biochip

Specific interactions were observed between calcineurin and GST-calmodulin spots whereas only background levels were detected on the GST spots. So the negative control (GST spot) data were subtracting.

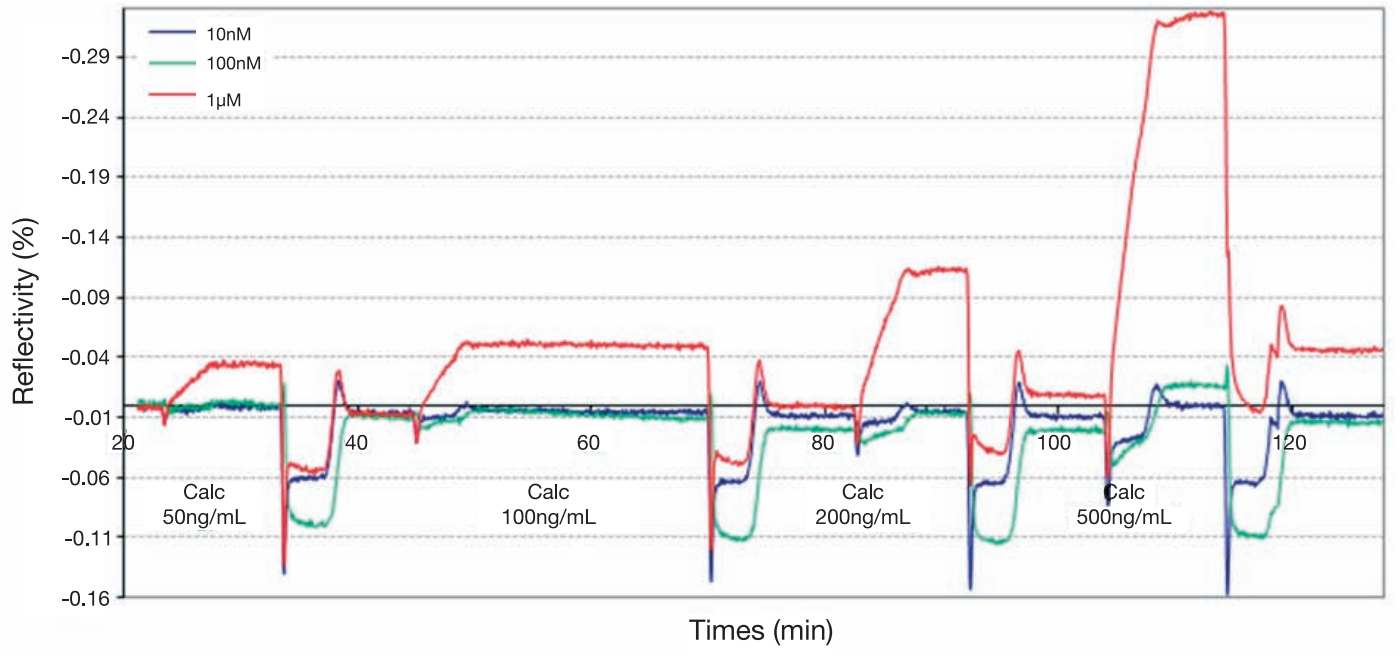

Figure 2: Average interaction curves on GST-CaM1 spots following calcineurin and EDTA injections.

Curves shown figure 2 indicated the average interaction for each GST-CaM1 spotting family. The red curve corresponding to the 1µM GST-CaM1 family displayed the higher interaction with calcineurin. No interaction could be observed for lower spotting concentration. Rapid association rate and low dissociation rate were observed.

Between proteins injection the chip was regenerated by EDTA injected solution. The signal returned to the previous baseline indicating a well complex denaturation (Figure 2).
The amount of calcineurin bound onto GST-CaM1 spots increased with the increasing protein concentration solutions injected (Figure 3). Calcineurin was significantly detected at 100ng/mL.

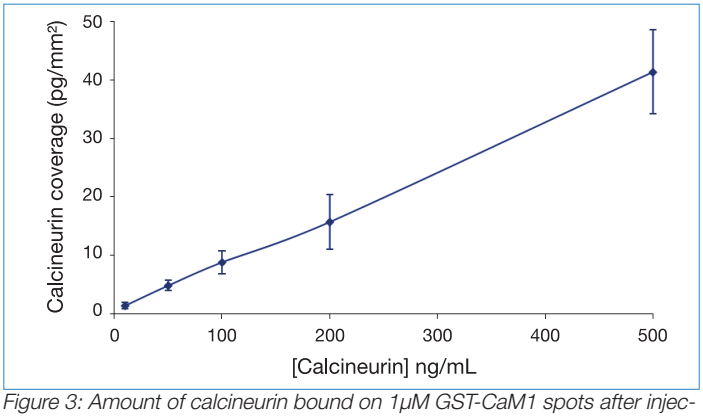

Figure 3: Amount of calcineurin bound on 1µM GST-CaM1 spots after injections of increasing concentration of calcineurin.

The SPR imaging technology allowed the real time visualization interaction of each spot during the experiment. Figure 1B showed an image during the injection of calcineurin. Only 1µM GST-CaM1 spots were visualized corresponding to high and specific proteins interaction.

Conclusion

We presented here a GST-derivative surface chemistry ready for partner GST-labeled probe immobilization. We showed that anti-GST covalently linked on cystamine/glutaraldehyde functionalized biochip surface is thoroughly suitable for GST-tagged probe immobilization. Moreover with SPRi technology in only one experiment a multiplex protein-protein interaction study was obtained. We followed in real-time without labeling specific and sensitive interaction. We determined the best spotting concentration of the immobilized partner. High throughput interactions were possible as complete biochip regeneration without partner activity loss could be obtained.

SPRi analysis technology and functionalized SPRi Biochip™ enable precise studies of GST-tagged protein/protein interaction, within a multiplex format, in real-time and without labeling.