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Application Note

Biology
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SPRi

SPR27

# Interaction between immobilized peptides and protein from sera on Cystamine / Glutaraldehyde functionalized biochip

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**Our SPRi technology (Surface Plasmon Resonance imaging), is able to measure multiplexed label-free biological interactions in a complex media such as serum.**
**We will demonstrate the ability to monitor specific interactions between immobilized peptides and proteins issued from non purified immune sera, using a cystamine/glutaraldehyde SAM surface chemistry on the biochip surface.**
**We chose to illustrate this ability by monitoring the interaction between an ovalbumin peptide fragment and an ovalbumin immunized serum.**

## Materials and methods

### Cystamine/Glutaraldehyde SAM biochip preparation

Briefly, the biochip surface was cleaned by UV-Ozone treatment, then immediately immersed in a cystamine/ethanol solution. The biochip was washed and dried and finally dipped into a glutaraldehyde solution, rinsed, dried and stored at 4°C before use.

### Multiplexed immobilization of peptides

The multiplexed peptide immobilization was performed directly on the functionalized SPRi-Biochip™ surface before introducing the chip into the SPRi system. The spotting solution (10 mM PBS with 10% glycerol) contained 25, 50, 100 or 200 µM of ovalbumin-peptides or casein peptides. Each peptide was used as the negative control of each other.
For the four different concentrations of each species, eight replicate spots were made.
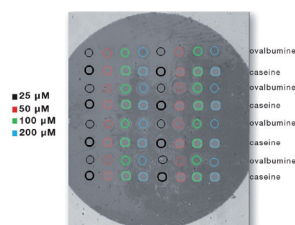


*Figure 1: SPRi image of spotted peptides.*

### SPRi experiment initialization

The biochip surface was blocked all around the immobilized peptide spots by the injection of a 10 mM glycine solution. Then, non specific sites were saturated using a 1% BSA solution.

## Injected solutions

200 µL of non-purified immune against ovalbumin or against casein serum solutions were injected at different dilutions, from 1/1 500 to 1/100, in the running buffer (10 mM PBS). A 100 mM Glycine/HCl (pH=2.0) solution was used to regenerate the interactions. Non-immune sera diluted at 1/100 in the running buffer was also injected as negative control.

## Results and discussion

Several solutions of ovalbumin immune sera from the lowest to the highest concentration were injected. Alternatively after each sample injection, a regeneration step was performed. The signal obtained on the negative control spots was subtracted from the positive signal.
Figure 2 displays the results obtained for the ovalbumin-peptide spots. As shown on the kinetic profiles, the specific interaction between the ovalbumine immune serum and the peptide increased with the concentration of analyte. High specificity is obtained as no interaction signal was observed between peptide spots and non-immune serum.
Figure 3 compares the average signal obtained for each ovalbumin-peptide concentration. The best interaction signal was obtained with peptides immobilized at 200 µM. This peptide concentration yielded a highly significant signal with immune serum diluted 1500 times.
This means that higher serum dilutions (thus less patient sample collection) could give meaningful results. This is an interesting result for applications such as the detection of low-abundant antibodies in serum.
Similar specific results were obtained for casein-immune sera injections on casein-peptide spots (data not shown).

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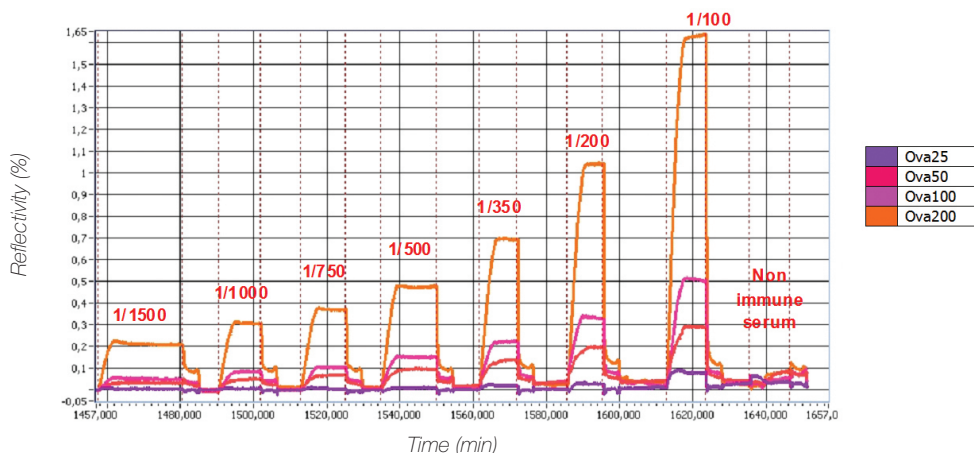
Figure 2: Average signal (reflectivity %) on ovalbumin-peptide spots after injections of ovalbumin immune sera solutions with decreasing dilution and non-immune serum.
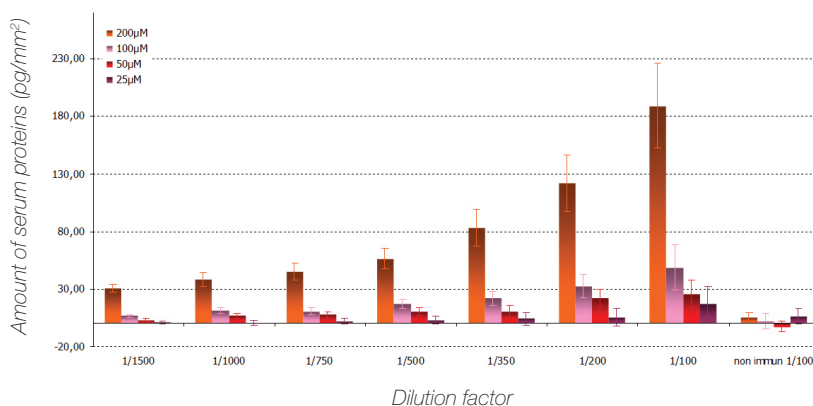


Figure 3: Protein amounts bound to ovalbumin-peptide spots after injections of ovalbumin immune sera solutions with decreasing dilution and non-immune serum.

## Conclusion

Biochips functionalized with Cystamine/Glutaraldehyde layers combined with the HORIBA Scientific SPRi technology allow a good specific interaction detection and quantification of targets contained in complex media like highly diluted sera.

In this application note, multiplexed peptide receptors can easily be immobilized on the biochip surface and peptide-protein interaction monitoring is made possible.

The surface chemistry developed by HORIBA Scientific greatly facilitates the direct immobilization of multiple receptor types on the SPRi-biochip™ surface. The biochip thus produced is resistant to hundreds of regenerations (Application Note 3222).

The HORIBA Scientific SPRi technology combined with appropriately functionalized biochip is a powerful tool for clinical applications such as diagnosis.

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