SPRi-0809.8-v1.1

Application Note

Detection of Small Molecules on Pyrrole-Streptavidin Biochip

We provide high performance instrumentation based on Surface Plasmon Resonance imaging (SPRi) technology to the pharmaceutical industry, biotech companies and academic research laboratories.
In the process of measuring biological interactions by SPRi technology, there is a high demand for the detection of small molecules (molecular weight < 1000 Da). We show here that our technology is able to detect molecules with molecular weights as low as 244 Da.

Introduction

The biological interaction chosen for this experiment is the streptavidin / biotin model. Streptavidin conjugated to a pyrrole molecule is electro-copolymerised on the gold surface of a biochip. The biotin is then captured on the biochip due to the strong streptavidin-biotin affinity

Materials and methods

SPRi-Biochip™ functionalization

Preparation and immobilization of pyrrole-streptavidin and pyrrole-mouse IgG conjugates

Streptavidin is conjugated with pyrrole-NHS in a phosphate buffer saline for 2 hours at room temperature. At the end of the process, pyrrole-streptavidin conjugate is desalted in a phosphate buffer containing 50 mM PO_4, 50 mM NaCl, 10 % glycerol.

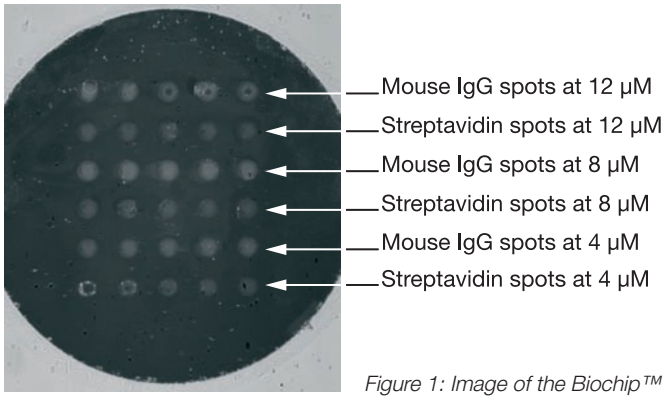

Figure 1: Image of the Biochip™

The negative control (mouse IgG) is conjugated with pyrrole-NHS in the same way as streptavidin.

The spotting solutions (phosphate buffer with 10 % glycerol) contained 20 mM free pyrrole and streptavidin or mouse IgG antibodies at 12 µM, 8 µM and 4 µM. The grafting of the biomolecules on the SPRi-Biochip™ was carried out by an electrochemical process. For polymerization of pyrrole-antibodies and pyrrole-streptavidin copolymer, an electrical pulse (2 V for 100 ms) was generated between the working electrode (prism gold surface) and the counter electrode (located in the arrayer pin). The tip was rinsed with distilled water after each spotting.

SPRi experiment

After its functionalization, the biochip was introduced into the SPRi instrument. The running buffer was 10 mM PBS.

Injected solution

Biotin, diluted at 20 ng/mL in the running buffer (10 mM PBS), was injected on the flow cell and interaction on the spots could be monitored in real time without labelling.

Results and discussion

SPRi quantification of small molecule binding on the biochip

Interaction curves

Figure 2 represents interaction curves on streptavidin

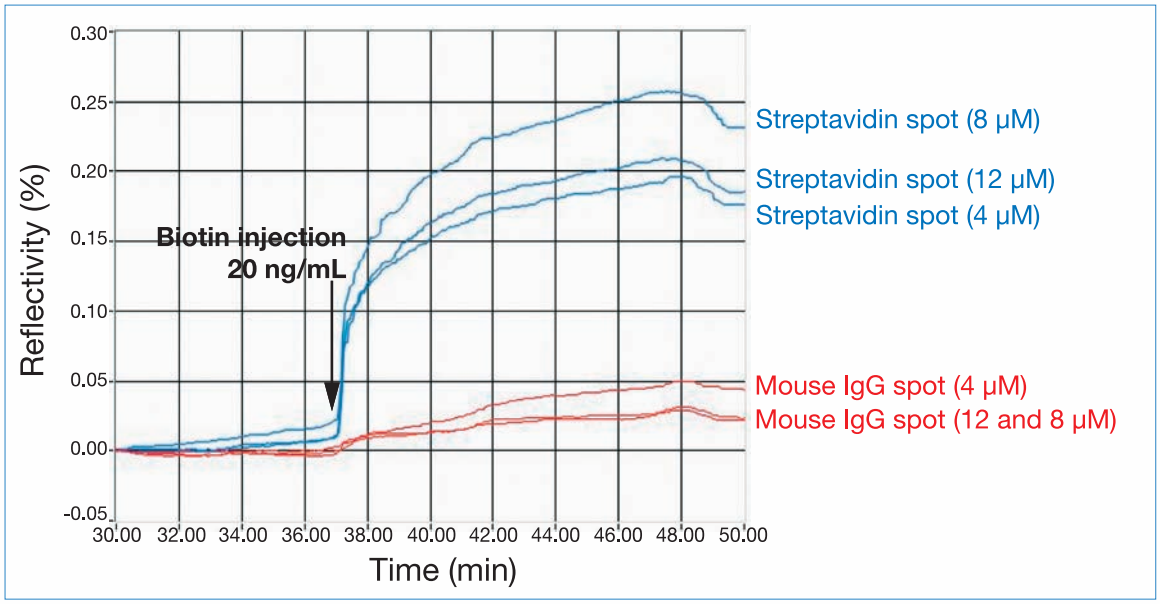

Figure 2: Interaction curves on streptavidin spots and on mouse IgG spots after injection of biotin

spots after injection of 20 ng/mL biotin.
Specific interactions are observed between streptavidin and the injected biotin whatever the spotting concentration of streptavidin. There is no significant interaction signal on the mIgG spots whatever their spotting concentration.
Streptavidin spotted at 8 µM seemed more sensitive than other streptavidin spots concentrations (4 and 12 µM) with only background being detected on mouse IgG spots.

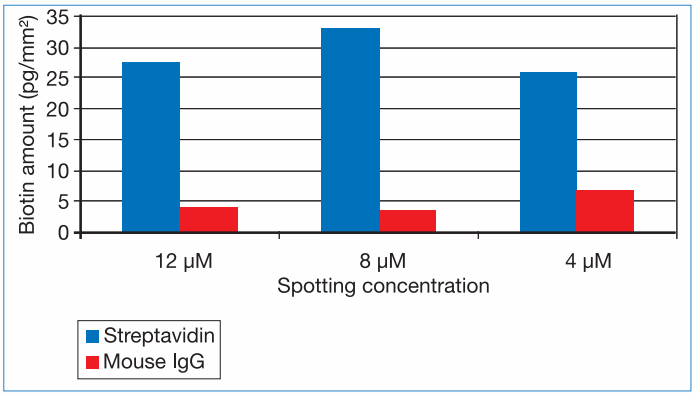

Figure 3: Amount of biotin on streptavidin and mouse IgG spots after injection of a biotin solution (0.02 µg/mL)

Protein amounts versus injection number

Histogram (figure 3) represents amount of biotin fixed on streptavidin and mouse IgG spots.
We observed that the amount of biotin on streptavidin spots varies from 25 pg/mm² to 32 pg/mm² i.e. between 19.5 pg and 25 pg by spot (spot diameter = 500 µm). The best spotting concentration of streptavidin is 8 µM.

Conclusion

We demonstrate here that the SPRi technology developed by Genoptics is thoroughly suited to detect small molecules, such as biotin, with molecular weights below 500 Da. Biotin captured on pyrrole-streptavidine spots and electro-grafted on SPRi-Biochip™ is specific and shows the high sensitivity of our SPRi technology instruments.