Peptide-Antibody Interaction Specificity

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GenOptics provides high performance instrumentation based on Surface Plasmon Resonance imaging (SPRi) technology to the pharmaceutical industry, biotech companies and academic research laboratories.

The study of the interaction between a peptide (1952 Da) and a monoclonal antibody can simply demonstrate the relevance of using SPRi technology for research.

First, streptavidin grafting is performed by using GenOptics pyrrole electro-polymerisation protocol. A mix of pyrrole and pyrrolated streptavidin is co-polymerised on the gold layer of a glass prism. Biotinylated peptides are then injected in the detection cell and create an homogeneous peptide layer, due to the strong affinity between streptavidin and biotin.

Experiment

A layer of streptavidin is deposited on the gold surface by electro-polymerisation of a pyrrole and pyrrolated streptavidin mix. The spotting concentration is 15 µM.

The concentration level of the injected peptides is 2 µg/ml. The antibody solution is injected at two concentration levels (0.1 and 0.3 µg/ml).

To ensure complete reproducibility, a regeneration step is run between each antibody injection.

The buffer used during this experiment is HEPES 10 mM, NaCl150 mM, EDTA 3 mM and 0.005% Tween 20.

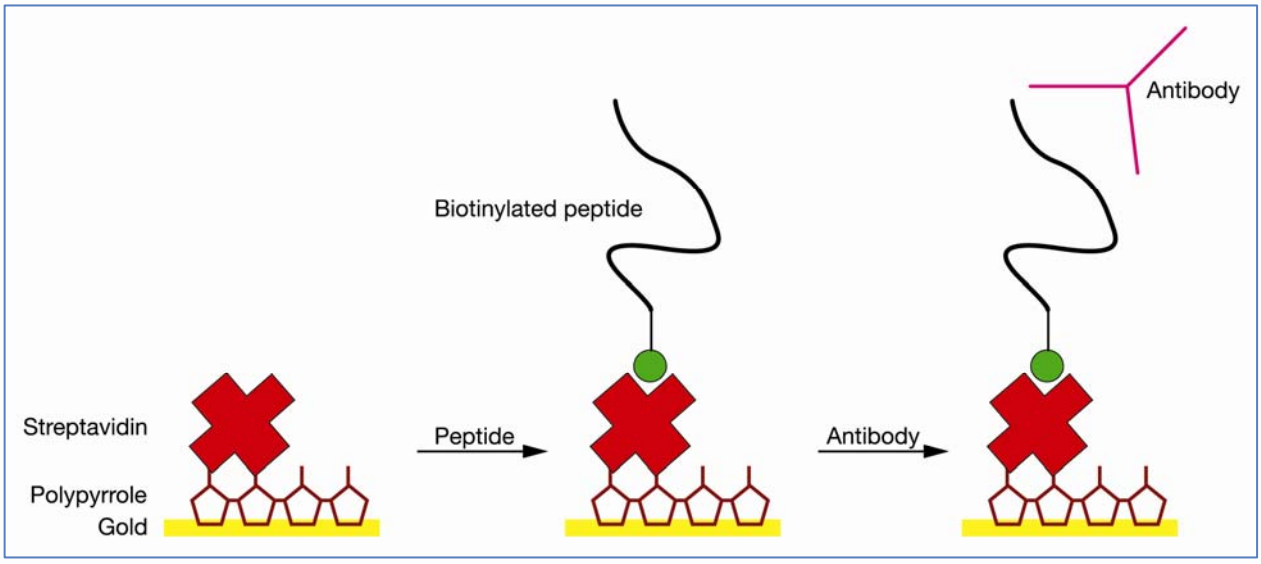

Fig. 1: Stages of the experiment

Peptide-antibody interaction

The kinetics of the interactions are shown on Figure 2. The upper line displays the collected signal from the peptide interacting with the antibody injected at different concentration levels.
The second signal is a negative control, showing no antibody interaction.

On a given spot, the reflectivity value becomes higher with the increased concentration levels of the injected antibody. It is easy to measure the affinity constant from the series of injected antibody concentrations. We determined a Kd value of 2.52 ± 0.79 nM. Data were obtained with a glass sensor prism exhibiting different spots densities. During this experiment the flow-rate is of 50 µl/min.

Note: Biotin injection (40 µg/ml) following the peptide injection allows surface blocking, which avoids a non-specific antibody adsorption.

The regeneration of the active surface of the glass prism can be carried out easily with a glycin pH 2.4, 100 nM/HCl buffer.

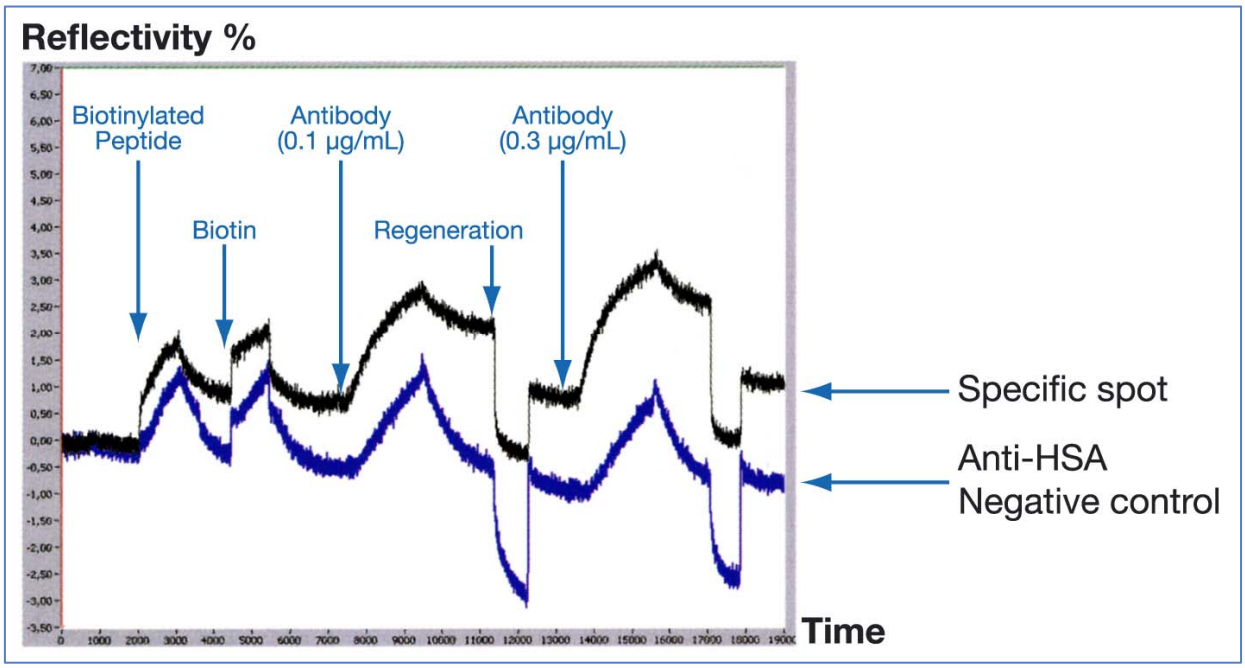

Fig. 2: Interaction kinetics between peptide and antibody (black) and on a non-specific spot (blue).

Conclusion

Antibodies are grafted on the surface of a gold layer using GenOptics electro-polymerisation technology. Peptides are injected at various concentrations allowing rapid determination of the affinity constants.

GenOptics SPRi technology enables precise studies of biomolecular interactions without labelling. The technique allows real-time studies of peptide-antibody interactions on a large number of spots analysed in parallel.