

Antibody-Antigen Specific Interaction

GenOptics provides the pharmaceutical industry, biotech companies and academic research laboratories with high performance instrumentation based on SPRi technology (Surface Plasmon Resonance imaging). To demonstrate the power of this technology, we carried out an experiment based on an antibody-antigen interaction. Original surface chemistry combined with an electrochemical process allows the rapid coupling of biomolecules to the gold layer (polypyrrole co-polymerisation) on the top of a glass prism.

Experiment

Principle

Two types of antibodies are first coupled to the pyrrole group and then grafted by electro-polymerisation onto the glass prism.

- Rabbit IgG (blue),
- Anti-hCG antibodies from mice (red).

Next, anti-rabbit IgG are added.

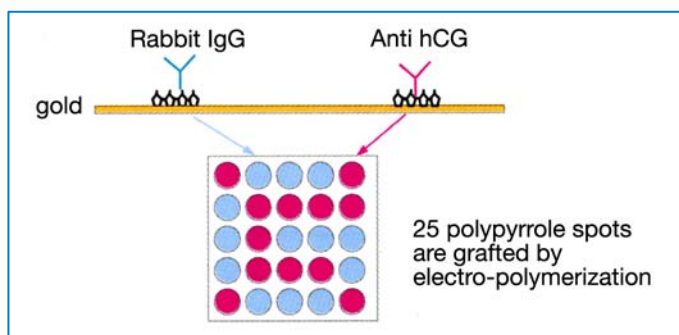


Fig. 1: Layout of a 25 spots matrix composed of rabbit IgG and anti-hCC IgG spots

Interactions between rabbit IgG and anti-rabbit IgG from goat

In the first experiment, anti-IgG from rabbit are injected. Figure 2 represents the kinetics of interaction between the antibodies immobilized on the gold surface and the anti-rabbit IgG injected in the detection cell.

There is a clear interaction between the rabbit IgG (red curves) and injected antibodies. There is no reaction with other spots. The interaction rabbit IgG and anti-rabbit IgG from rabbit is specific and can be monitored on a real time basis.

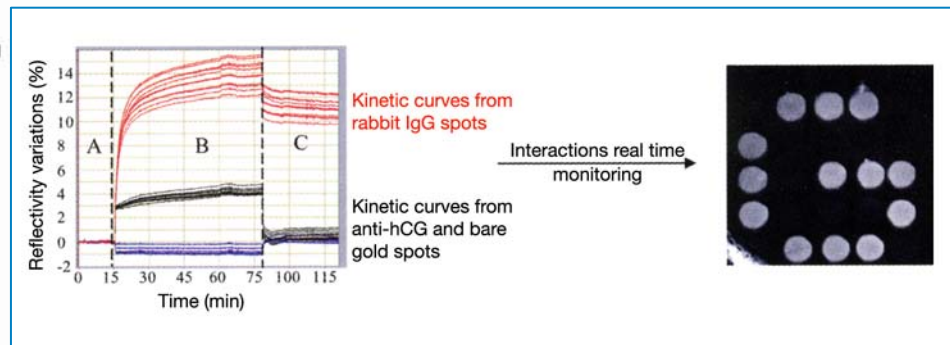


Fig. 2: Reflectivity variations as a function of time following injection of anti-IgG from rabbit
 A: PBS/Tween buffer
 B: Injection of the anti-IgG
 C: Rinsing with PBS/Tween buffer

Example of interactions: hCG - mouse anti-hgG, hCG - rabbit anti-hCG, rabbit anti-hCG - anti-rabbit IgG

In a second experiment, a mouse anti-hCG antibody is firstly immobilised on the gold surface of the glass prism. The surface is then successively exposed to various solutions containing hCG, anti-hCG antibodies (different from those already immobilised), and a rabbit anti-IgG antibody.

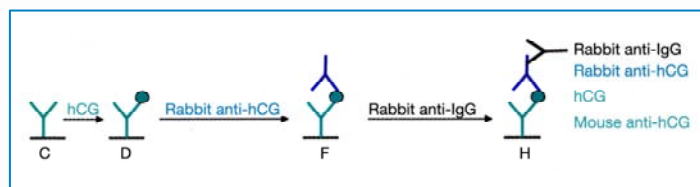


Fig. 3: Interactions between a mouse anti-hCG antibody and hCG (D), then between hCG and a rabbit anti-hCG (F), and between a rabbit anti-IgG antibody and an anti-rabbit IgG antibody (H).

The graph below shows these different interactions monitored in real time:

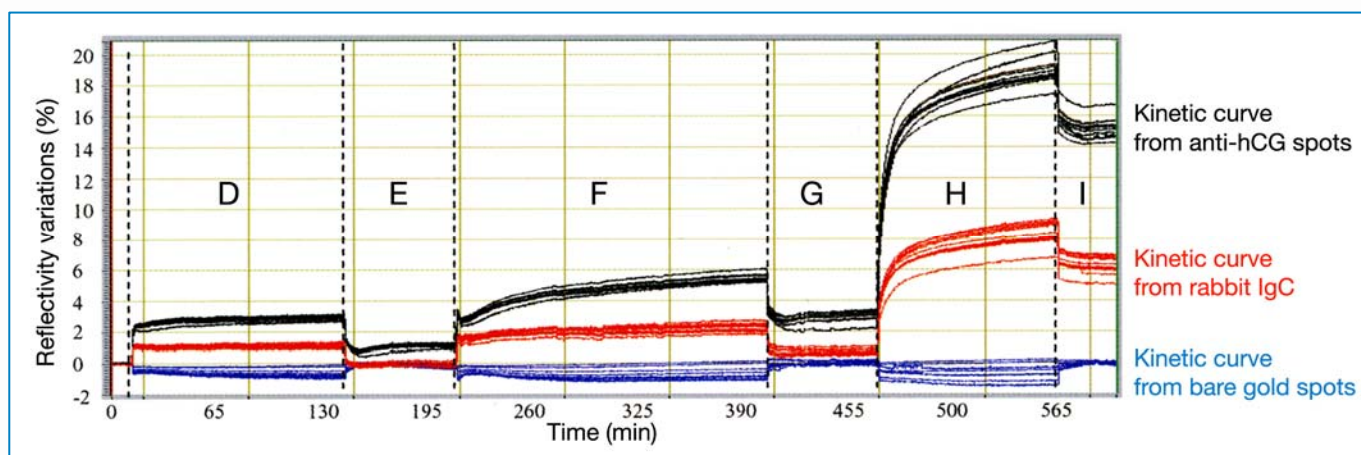


Fig. 4: Kinetic curves on mouse anti-hCG spots, rabbit IgG and bare surface, following subsequent injections of hCG, rabbit anti-hCG and rabbit anti-IgG.

E, C, I: Injection of PBS 10 mM, Tween 0.05

D: Injection of 250 nM hCG

F: Injection of anti-hCG (monoclonal antibody 210 nM),

H: Injection of goat anti-rabbit antibody (polyclonal 220 nM)

Conclusion

GenOptics SPRI technology allows a real time study of biomolecular interactions. No additional labelling reagent is required, and a large number of spots can be analysed at the same time and in the same condition.

This technique is therefore proven to be efficient at performing accurate high throughput antibody screening.