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Application Note

Clinically related protein-peptide interactions monitored in real time on novel peptide chips by Surface Plasmon Resonance Imaging

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Context and objectives

Proteins constitute the essential machinery in cellular life; almost all cellular responses are triggered by protein-protein interactions. Performing assays to specifically analyze binding phenomena is important for understanding cellular mechanisms and for designing pharmacologic targets. These events cannot be studied with RNA/DNA chips because RNA concentrations do not always correlate with protein production and/or activity.

Protein array techniques have recently emerged but these arrays remain difficult to construct because of the complexity and heterogeneity of proteins and the necessity to preserve their conformational folding after covalent binding to a surface.

Microarray technology has a crucial role to play in detecting molecule-molecule interactions. The use of peptide chips with indirect detection by use of labelled probes allows qualitative analyses of samples but fails to provide quantitative data such as kinetics.

Optical detection by surface plasmon resonance (SPR) is an accurate, one-step method for the real-time direct measurement of ligand binding without labelling. However, this approach does not allow for parallel analysis on a single chip bearing an array of proteins or peptides.

The aim of this application note is to describe the advantages of combining the use of peptide chips with direct label-free detection as achieved by SPR imaging (SPRi).

Description of the experiment

Eleven peptides derived from hepatitis C virus (HCV) and one peptide from ovalbumin (negative control) were synthesized with a maleimide-modified NH_2 terminus and were conjugated with SH-activated pyrrole. Then, the pyrrole-peptide conjugates were electro-deposited on the gold layer of the chip.

The biochip was then introduced in a GenOptics SPRi-Plex™ platform in order to study peptides-circulated antibody interactions in complex media such as undiluted sera using SPRi.

Results

Specificity of antibody binding to peptide chips and SPRi detection

Injection of an anti-C 20-40 immune serum gave to a specific signal corresponding to the spot bearing the C 20-40 peptide, whereas low signals were observed with the irrelevant C 131-150 and ovalbumin peptides as well as with the polypyrrole alone. However, injection of non-immune serum led to a low and similar reflectivity from all of the spots which correspond to non-specific binding. Subsequent injection of the anti-C 131-150 immune serum confirmed the specificity of the method.

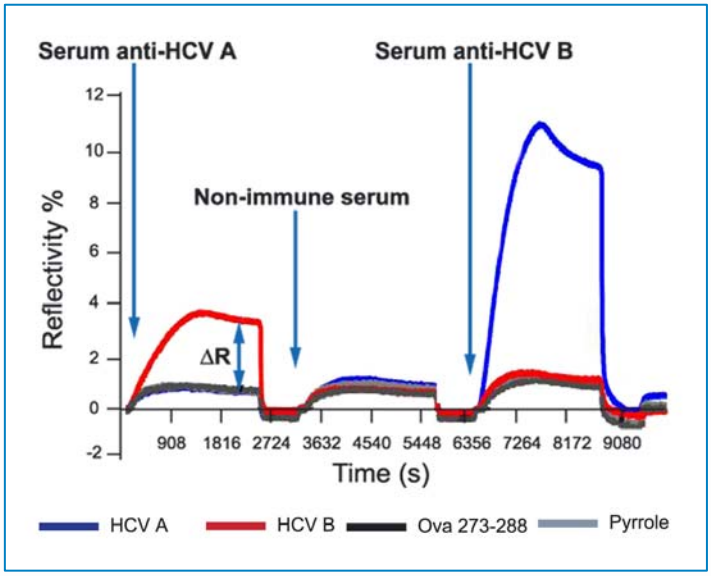
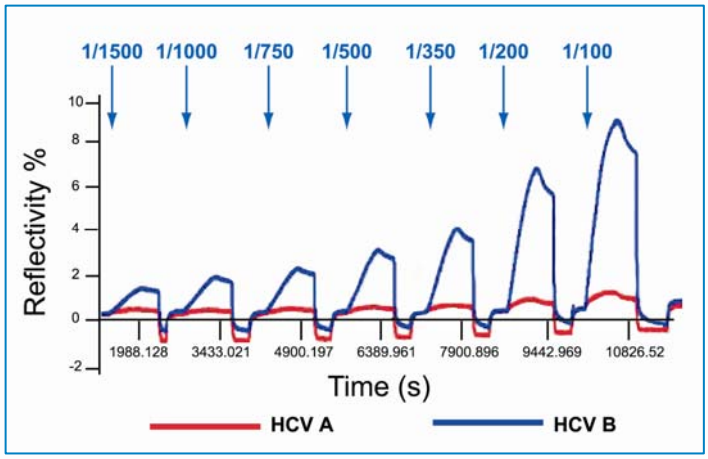


Figure 1: Specificity of antibody binding on peptide chips. Three peptides (C20-40, C131-150, and Ova 273-288) were spotted on the surface of a chip, and different rabbit immune sera were successively injected. The chip surface was regenerated after each assay

SPRi quantification of antibody binding on peptide chips



Serial dilutions of anti-C 131-150 antiserum were successively injected in the flow-cell. The value obtained at the plateau (8% of reflectivity) corresponds to a monolayer of antibody equivalent to 1 pmol/mm^2. One important application of this method is the detection of antibodies (or other molecules) present which are only present at low concentrations.

Figure 2: Quantification of antibodies bound to peptide chips Serial dilutions of anti-C 131-150 antiserum were successively injected. The chip surface was regenerated after each assay.

Analysis of patient-derived sera by use of peptide chips and SPRi detection

Eleven sera samples from HCV-positive patients and from healthy donors were successively injected on a chip bearing 11 HCV peptides. The specific SPRi signal (ΔR) varied according to the patient and the peptide, revealing donor-related differences in the anti-HCV antibody responses (specificity, amount and affinity).

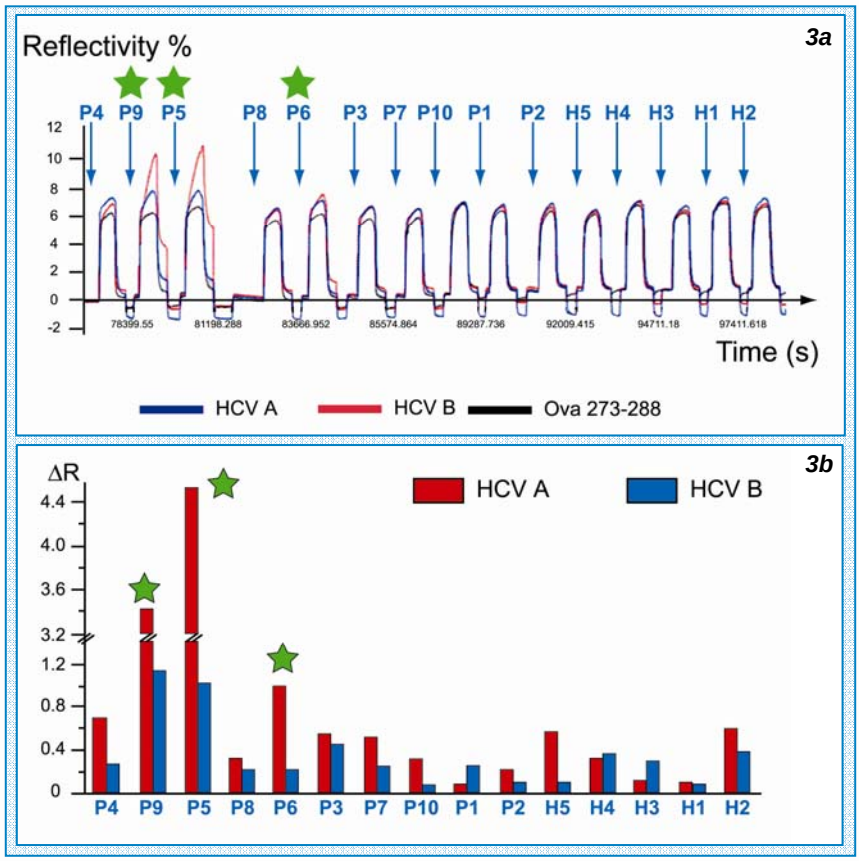


Figure 3:
SPRi analysis of sera from 10 HCV patients (P1-P10) and from 1 healthy donor (H1). Injection of human sera on a chip bearing 11 HCV peptides and Ova (negative control). The chip was regenerated between 2 successive injections.
***3a**: Interaction curves obtained after injection of the 11 sera samples. **3b**: ΔR for each serum.*

Conclusion

The GenOptics platform, centred on SPRi technology, enables:
- The generation of biochips bearing a large range of peptide probes (peptide arrays)
- The label-free detection of peptide-antibody interactions in complex media such as undiluted sera

The GenOptics SPRi technology is a powerful tool for clinical applications such as diagnosis, pharmacologic studies, and for research.

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GENOPTICS BIO INTERACTIONS

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