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Application Note

SPRi / MALDI-MS Array Platform

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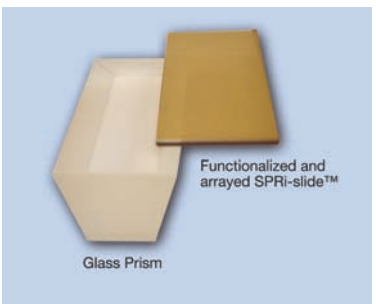
The combination of SPRi technology with MALDI mass spectrometry on the same surface is a powerful approach for the analysis of biomolecular interactions. Surface Plasmon Resonance is used to quantify interactions between capture ligands and surface-immobilized receptor combined with SPR, Mass Spectrometry (MS) enables the determination of the molecular weight of specifically bound ligands.
The SPR-MS system, implemented by GenOptics, allows the measurement of biomolecular interactions by SPRi, and then the detection of the retained ligands by MALDI mass spectrometry directly from the sensor surface. This system has been validated from an antibody-antigen biomolecular system as described below.

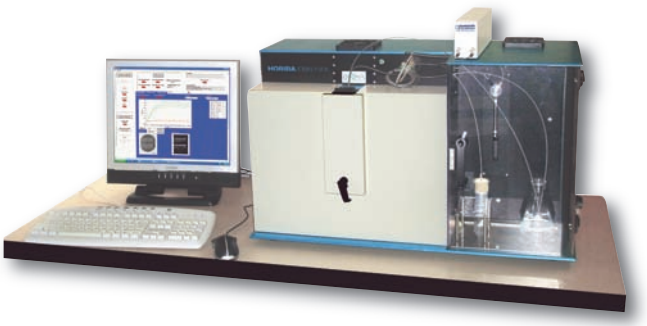
Experimental device

a) In this experiment an SPRi-slide™ was used as the biochip, the surface of which was functionalized by an NHS group. The SPRi-slide™ was placed onto a glass prism with an oil layer between the two ensuring good optical contact.



b) The SPR experiments were performed on a GenOptics SPR instrument (SPRi-Plex™ or SPRi-lab™).

c) Mass spectra were obtained using a MALDI mass spectrometer.



Protein immobilization on a NHS functionalized SPRi-slide™

The gold surface of the SPR sensor, the SPRi-slide™, was functionalized with the amine coupling reagent *N*-hydroxysuccinimide (NHS). The proteins used as receptors were covalently linked to the surface through their primary amine functions by being simply dropped onto the SPR sensor.

SPRi experiment

The SPRi-slide was placed on top of the glass prism and the biochip holder introduced into the SPRI-Plex™ system. An MS compatible running buffer was choosen, such as 10 mM ammonium acetate (pH7.5), and a blocking lysine solution injected into the system to react with the remaining free active NHS groups. The biochip was then ready for SPR and analyte capture experiments. A mixture of proteins diluted in the running buffer was now injected in the flow cell and the analyte capture was followed in real time by SPR imaging (Fig.1).

After the capture step (10 min), a washing step (10 min) was carried out with the running buffer in order to remove non-specifically bound proteins, as depicted in Fig. 2, for the interaction between antibodies and their antigens.

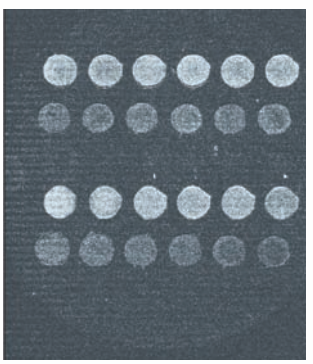


Figure 1:
Following the analyte capture in real time on the SPRi-Plex™

If several successive injections are required, a 100 mM glycine solution (pH 2) can be used as a regeneration solution.

The antigens specifically bound are removed and antibodies receptors are ready to interact with others potential analytes.

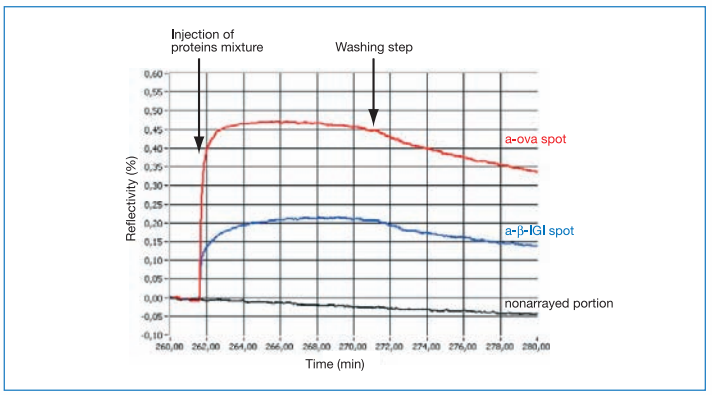


*Figure 2: **SPR analysis of interactions between a mixture of ovalbumin and β-lactoglobulin with their specific antibodies arrayed on the SPR sensor.** Reflectivity variations versus time of anti-ovalbumin (a-ova, 600 nM), anti-βIGI-lactoglobulin (a-βIGI, 600 nM) spots and of a nonarrayed portion of the chip (negative control) after injection of a 50 µg/mL mixture of β-lactoglobulin and ovalbumin*

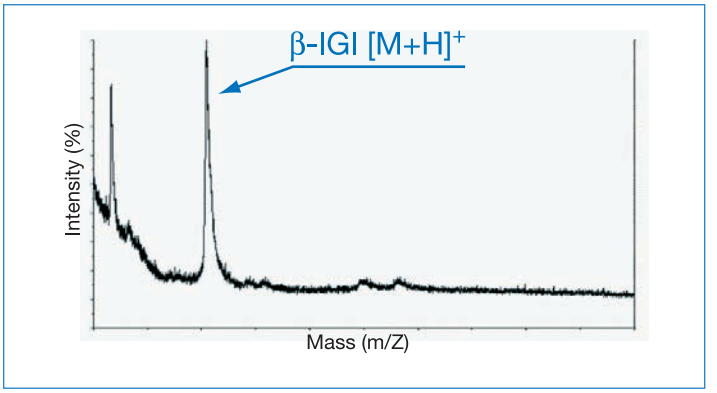


Figure 3: MALDI mass spectra obtained for β-lactoglobulin specifically captured on their antibodies spotted on the biochip, $[M+2H]^{2}+$ and $[M+H]^{+}$ of β-lactoglobulin.

Biochip transfer and matrix deposition

After SPR and analyte capture experiments, the biochip was removed from the SPRi-Plex™ and inserted directly into an appropriate MALDI-MS plate holder. Finally a MALDI matrix was dropped on each localized spot.

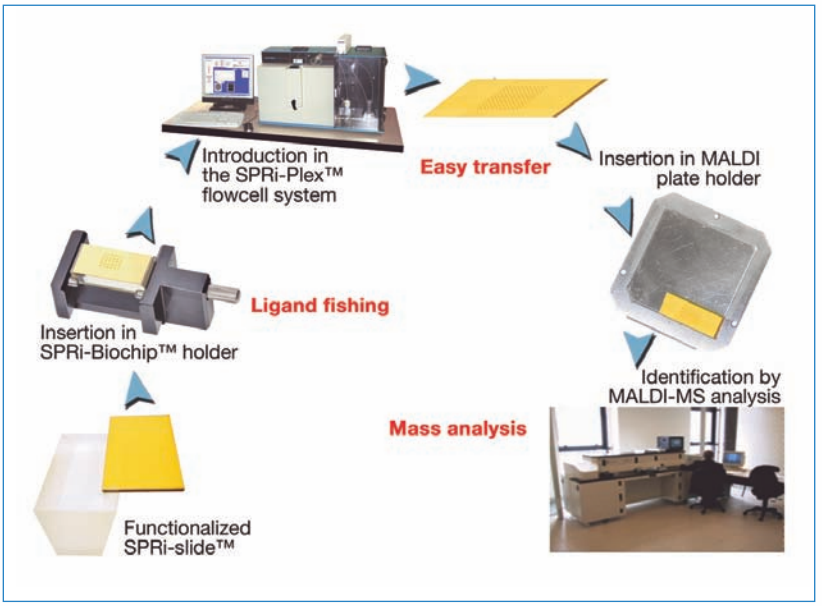
On-chip MALDI-MS experiment

Mass spectrometry was directly performed on the SPR chip following the affinity capture of the specific proteins, using a commercially available Applied Voyager MALDI-TOF instrument. Typical mass spectra obtained from the protein-bound antibody array show specific multiply charged protein ions.

The mass spectrum obtained from the protein-bound antibody array show specific multiply charged ions. The mass spectrum below corresponds to an analyte specifically retained on a-βIGI spot (Fig. 3). The presence of $[M+2H]^{2}+$ and $[M+H]^{+}$ ions reflects the capture of β-lactoglobulin and there is no trace of any non-specific binding being observed on this spot. The quantity The quantity of specific proteins being retained is enough to obtain a good quality mass spectrum. A mass spectrum is also acquired from the underivatized portion of the chip, allowing the control of the absence of non-specific binding of proteins on the chip surface.

Conclusion

The combination of SPRi with MALDI-MS on an array platform is a powerful tool for affinity separation and identification of specific ligands from complex solutions. A versatile sensor surface has been designed to allow SPR ligand fishing and MALDI MS identification of the retained ligand. SPRi-slide™ transfer from SPRi-Plex™ to MALDI-MS plate is easy and MS analysis is directly carried out on the micro-array biochip. High-throughput multiplexed analysis with spatial resolution is permitted with this array platform.



Versatile SPR/MS micro-array platform

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