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Surface Plasmon Resonance imaging

Label-free Ligand Fishing in Human Plasma Using Surface Plasmon Resonance and Mass Spectrometry imaging

Application Note
Biology
AN SPRi 34

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Surface Plasmon Resonance (SPR) is an optical technique that offers label-free biomolecular analyses, providing information on kinetic processes (association and dissociation), binding affinity, analyte concentration and real time molecule detection. It has become a powerful tool for the analysis of biomolecular events involved in drug development, cancer research, and antibody screening, …

The phenomenon of SPR occurs when light interacts at the interface between a biochip and a liquid medium. It permits to follow modifications of the refractive index (or the reflectivity) in real time. Such modifications are induced by a biomolecular interaction between immobilized ligands (probe molecules) and captured analytes (target molecules). SPR monitors theses changes of reflectivity to characterize the biomolecular events (such as binding and dissociation) occurring at the surface of the biochip in real time.

Surface Plasmon Resonance imaging (SPRi) technology offered by HORIBA Scientific takes SPR analysis a step further. The OpenPlex™ and XelPlex™ instruments (Figure 1) enable visualizing the whole biochip surface in real time using a video CCD camera.

This design allows biochips to be prepared in an array format; with each spot corresponding to a specific immobilized ligand. Up to several hundreds different molecules can be spotted using an automated spotter, opening the way to high throughput information for biomolecular interaction. The multiplexing capabilities of SPRi can meet with any experimental design without concession. Meanwhile, the sensitivity of SPRi is not compromised as analyte concentrations can be detected down to the nanomolar range.

Figure 1: HORIBA Scientific XelPlex™ system

The applications of SPRi are vast and include for example protein:protein[1], DNA:DNA[2,3], peptide:protein[4], polysaccharides:proteins[5] or protein:cells[6,7] interactions.

The flexibility of the HORIBA Scientific instruments enables complex samples such as serum and plasma to be analyzed for clinical applications.

General Overview of SPRi and Mass Spectrometry coupling

The coupling of SPRi biosensors and matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) is an innovative approach for biomarker discovery in biological fluids. It permits analytes captured by SPRi to be identified and characterized by their molecular weight and peptide sequence. SPRi-MS opens a new method of detection, quantification and structural characterization of proteins of interest. In the future, it could help better discriminate between sub-species within a family of biomarkers.
In this context, the complexity lies in the coupling of both techniques[8]. Most strategies require the elution of the bound analyte and its analysis by ESI- (electrospay ionisation) or MALDI-MS. This procedure has many drawbacks (analysis time, no multiplexing capabilities, decreased sensitivity, additional cross-contamination risks, etc…) which delayed the development of SPR-MS in the diagnostic field.

The open format of the HORIBA Scientific instruments makes MS coupling easier and faster. The possibility of direct MS analysis on the SPRi sensor was recently shown[9]. The SuPRa-MS platform (Surface Plasmon Resonance in arrays coupled with Mass Spectrometry) combines SPRi and MS in a single biochip. The biochip used for SPRi (SPRi-Slide) is directly transferred to the MS instrument. There is no need to neither elute nor re-deposit the bound analyte. The MS enzymatic digestion and the deposition of the MALDI matrix are performed directly on the SPRi-Slide. The latter is then directly placed on the MS plate holder (Figure 2).

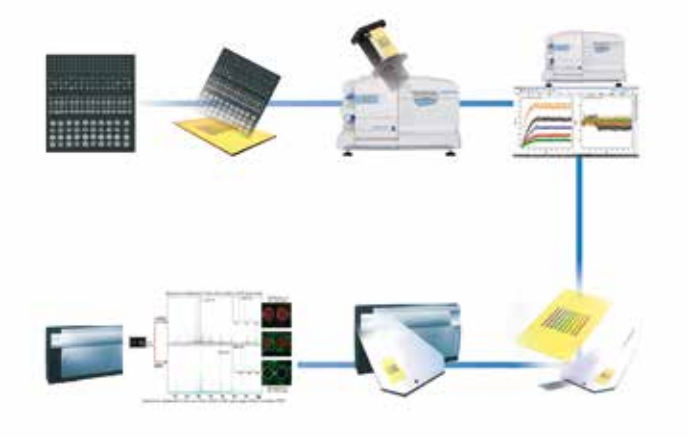

Figure 2: The SuPRa-MS platform

Biomarker Capture and Identification using the SuPRa-MS Platform

A proof-of-concept study of SPRi-MS imaging coupling was performed for the detection of LAG3 recombinant protein in plasma. The solution fraction of this protein is a potential biomarker for breast cancer[10]. For this purpose, a mouse antibody (IgG2A) directed against LAG3 was immobilized on a SPRi-Slide using a dedicated surface chemistry compatible with MS analysis (NHS chemistry). Before injecting LAG3, rat serum albumin (RSA) was used to avoid non-specific binding on the surface of the biochip. Then, the specific interaction of LAG3 (added in plasma) and IgG2A was monitored using SPRi and images of the interaction were studied. Several femtomoles/mm^2 of LAG3 proteins were captured by SPRi. After direct processing on the biochip surface (enzymatic digestion and matrix deposition), the SPRi-Slide was analyzed using a MALDI-MS imager (Ultraflex, Bruker Daltonics). By showing the distribution of MS peaks specific of LAG3 and RSA respectively, it was possible to build the MS image of LAG3 spots (Figure 3) directly on the SPRi-Slide.

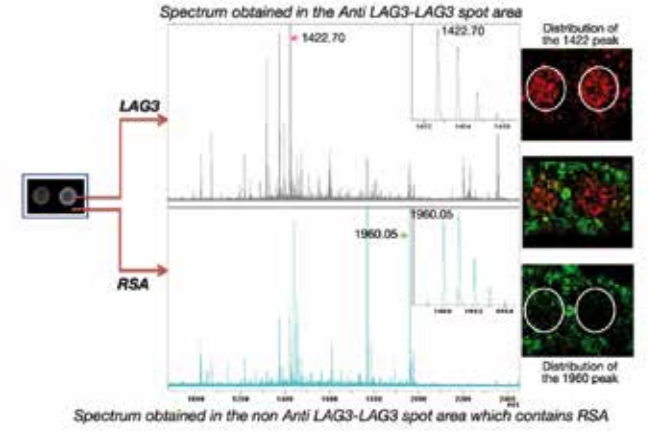

Figure 3: On-a-chip detection, identification and imaging LAG3 protein (potential marker of breast cancer) at 10 nM in human plasma through the SupRA-MS platform

The SuPRa-MS platform pioneers the combination of SPR imaging and MS imaging (MSi). It offers the possibility to gain spatially resolved information on the capture, sequence and molecular weight of clinical biomarkers.

Conclusion

Multiplexed SPRi analysis using the HORIBA Scientific instruments provides rapid and high-throughput information in real time from up to several hundreds interactions in parallel. The technology is sensitive and does not require the use of labels. It can speed-up the workflow and reduce consumable costs during optimization processes. The coupling with MS analysis is straightforward and easier, which makes it a valuable tool for biomarker identification.

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