Antibodies Exploration Thanks to Label-free Surface Plasmon Resonance Imaging Technology

Surface plasmon resonance imaging allows molecules binding study in label-free and real-time conditions. The power of the technique comes from its singularities, which are multiplexing and imaging, leading to a considerable speed-up of the processes' analyses. This will be illustrated for antibody molecule study by three applications.

The first application aims to validate the sensitivity of the SPRi technology for the detection of small molecules. To do so, a monoclonal antibody highly specific to a steroid hormone (undisclosed) of 290 Da was analysed. This monoclonal antibody was developed at SynAbs using a new hybridoma technology for guinea pig mAb and was rescued and produced by Diaclone.

In the antibody production processes, the final step is the characterisation of antibodies. Determination of immunoglobulin isotype is one part of the characterisation procedure. This second application demonstrates SPRi technique can be used to determine immunoglobulin isotype. Different rat monoclonal antibodies specific to different isotypes, produced by SynAbs, were immobilised on a functionalised SPRi-Biochip™. In this specific application, we highlight the screening capability of the SPRi technique, where different antibodies at different conditions can be analysed simultaneously and in a single biochip.

Finally, the third application focuses on the experiment conditions optimisation. It shows how single-domain antibodies can be easily studied with SPRi technology. QVQ develops custom made single-domain antibodies from camelids (VHH), which can be directed towards specific epitopes and have similar high affinity and specificity as compared to conventional Abs.

Surface Plasmon Resonance Imaging Platform (Figure 1)

In each application, antibodies or single-domain antibodies were first immobilised on SPRi-Biochips™-activated surface using contact spotting (SPRi-Arrayer™) and flow printing (SPRi-CFM).

The SPRi-Arrayer™ is an automatic and compact system for immobilising ligands in a multiplex format onto a SPRi-Biochip™ or a SPRi-slide™. This versatile instrument uses direct contact spotting and is suitable for printing on bare or 2D-functionalised SPRi-Biochips™ or SPRi-Slides™. Contact spotting allows fast and flexible microarray printings. The diameter of the printing pin can be adapted to the number of required spots in the matrix. Here, the diameter of the printing pin was 500 µm.

The SPRi-CFM uses continuous flow deposition to immobilise up to 48 molecules in a single printing run. Three printing runs can be performed on a single biochip (and up to 144 spots per chip can be generated). The microfluidic immobilisation improves the spot homogeneity and gives a higher immobilisation level. For this experiment, the flow rate of the SPRi-CFM was set to 15 µL/min and the contact time to 30 minutes.

The printed SPRi-Biochips™ were then loaded into the XelPleX system. The interactions were monitored using EzSuite software. The running buffer was 10 mM PBS pH 7.4 and the working temperature was set to 25°C.

Then, 200 µL of the studied molecules were injected into the fluidic system at a flow rate of 50 µL/min. A regeneration cycle was performed between each analyte injection by flowing either a 0.1 M glycine-HCl pH2.0 solution or a 10 mM NaOH solution with contact times between 30 seconds and four minutes.

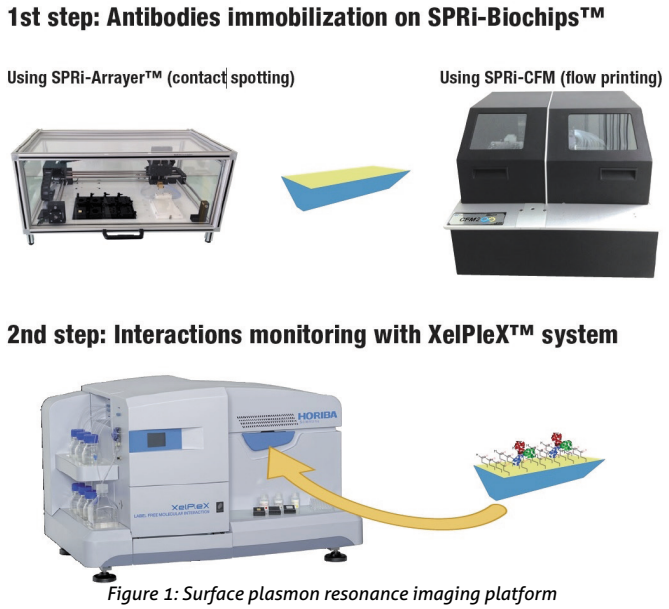

Figure 1: Surface plasmon resonance imaging platform

Application 1: Steroid Hormone Detection

A monoclonal antibody (mAb) developed at SynAbs by a new hybridoma technology for guinea pig mAb was rescued and produced by Diaclone. That monoclonal antibody binds specifically to a 290 Daltons steroid hormone (undisclosed).

The hormone was injected at five increasing concentrations following a three-fold dilution series from 1.5 to 125 nM.

The SPRi kinetic curves were analysed using the EzFit software to determine the kinetic constants and to calculate the affinity. This software is suitable for processing multiplexed data intuitively. The SPRi signal obtained on reference spots (i.e. Diaclone negative control antibody) were used for referencing. Then, the data were fitted locally (i.e. Rmax "maximum of reflectivity" different for each curve) using a 1:1 interaction model (see Figure 2; orange curves correspond to the fits).

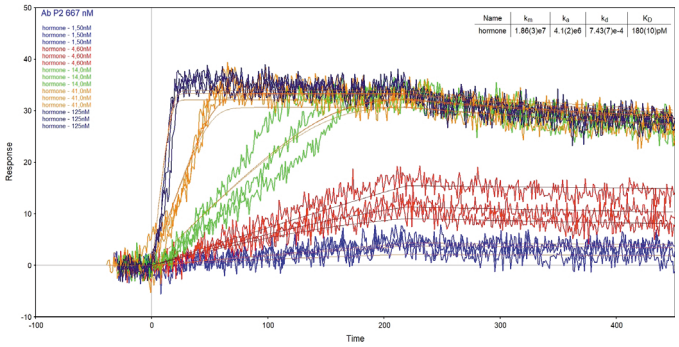

Figure 2: Kinetic analysis of the antibody / hormone interactions (local fits using a 1:1 interaction model)

The limit of detection of the hormone by the guinea pig antibody was determined to be around 1.5 nM (~ 0.45 ng/mL). This limit of detection was obtained with a flow printing of the antibody on a 3D chemistry (dextran-based chemistry).

It's the same limit of detection as that of a conventional ELISA technique. ELISA is the standard technique used in the validation process of Diaclone.

The kinetic curves profile showed mass transport limited kinetics. An affinity of 0.2 nM for the hormone / antibody interaction was calculated with this mass transport effect taken into account in the EzFit software by the addition of a km constant.

The specificity of the antibody was also verified by flowing an analogue hormone at the same concentrations. No binding response was observed with the antibody of interest while injecting the analogue hormone.

Thanks to the array-based format of the SPRi sensor chips, it is easy to extend these results to multiple interactions and to quickly integrate the XelPleX system into biomolecule production processes such as Diaclone's monoclonal antibodies production process at the validation step.

Application 2: Determination of Immunoglobulin Isotype

Antibody isotyping consists in determining a monoclonal antibody class and subclass identity. Isotyping is a common basic test done in any immunological research and clinical diagnostics lab that requires antibody production. Determining the class and subclass identity of an antibody is a critical and valuable characteristic for functional activity, purification strategies, use in immunoassays and long-term stability.

SPRi technology was tested to show how it can extend isotyping to multiple monoclonal antibodies produced.

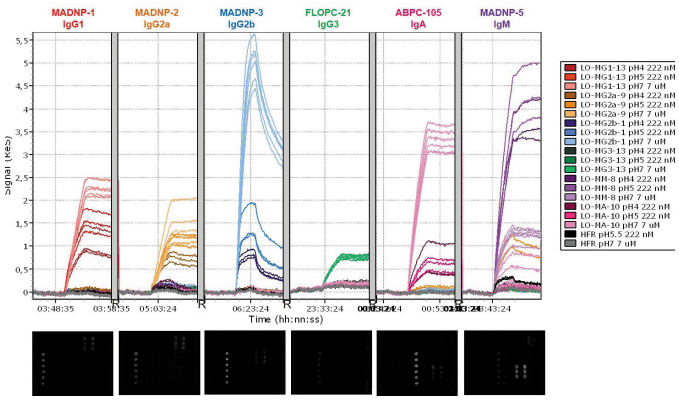

Figure 3: Global overview of raw kinetic curves: one curve for one spot, after successive injections of the six mouse mAbs at 1 µg/mL and corresponding difference images below each mouse mAb injection.

The test consisted in an analysis of six different mouse mAbs from SynAbs immobilising six different rat anti-mouse isotype mAbs from SynAbs.

Figure 3 shows a global overview of raw kinetic curves per spot obtained during the SPRi experiment after successive injections of the six mouse mAbs (MADNP-1, MADNP-2, MADNP-3, FLOPC-21, ABPC-105 and MADNP-5), each prepared at a concentration of 1 µg/mL. Regeneration steps (R) between each mouse mAb injection are not shown (light grey strips R identified). Each injection kinetic is associated to a corresponding difference image saved during the dissociation phase.

As each rat anti-isotype mAb was immobilised at three different pHs and in replicates (three or six according to immobilisation system), 81 different curves in all are represented for each mouse mAb injection.

Isotypes of mouse mAbs injected can be easily determined by looking at the raw kinetic curves of Figure 3 as well as the difference images. Difference images especially give a quick yes/no binding answer. Thus, if we go into more detail:

MADNP-1 is an IgG1 isotype;
MADNP-2 is an IgG2a isotype;
MADNP-3 is an IgG2b isotype;
FLOPC-21 is an IgG3 isotype;
ABPC-105 is an IgA isotype;
MADNP-5 is an IgM isotype.

The mouse mAb concentration of injection was set at 1 µg/mL for the SPRi experiment as it is the minimal standard concentration used in isotyping kits. This concentration was completely suitable for a SPRi experiment. Indeed, isotype determination was greatly reliable for each mouse mAb tested.

Exploiting at its maximum the wide printing surface of SPRi-Biochips™ and the multiplexing particularity of SPRi systems, one can easily imagine an isotyping analysis at a higher throughput level.

Application 3: Single-domain Antibody Study

Single-domain antibodies from camelids (sdAbs, VHH also referred to as Nanobodies®) are considered as the "third generation" of antibodies after conventional monoclonal antibodies (mAbs) and antibody fragments (Fab and scFv)[1]. They consist of single monomeric variable antibody domains. Because of their small size, they differentiate with several advantages of interest in medicine (enhanced tissue penetration, rapid clearance) and in biotechnology such as biosensors areas (higher immobilisation potential and enhanced detection sensitivity, superior stability)[2].

Q17c, a commercial sdAb from QVQ, recognises specifically HER2 recombinant protein, one of the epidermal growth factor receptors expressed in breast cancer.

Q17c protein was immobilised under nine different conditions, i.e. using the two different immobilisation systems, prepared at three different pH levels (4.0, 5.0 and 7.4) and at six different concentrations (167, 333 and 667 nM with fluidic printing and 1.75, 3.5 and 7 µM with contact spotting) on a single biochip.

The large working area of the SPRi-Biochip™ and the multiplexing capabilities of SPRi systems allow for the

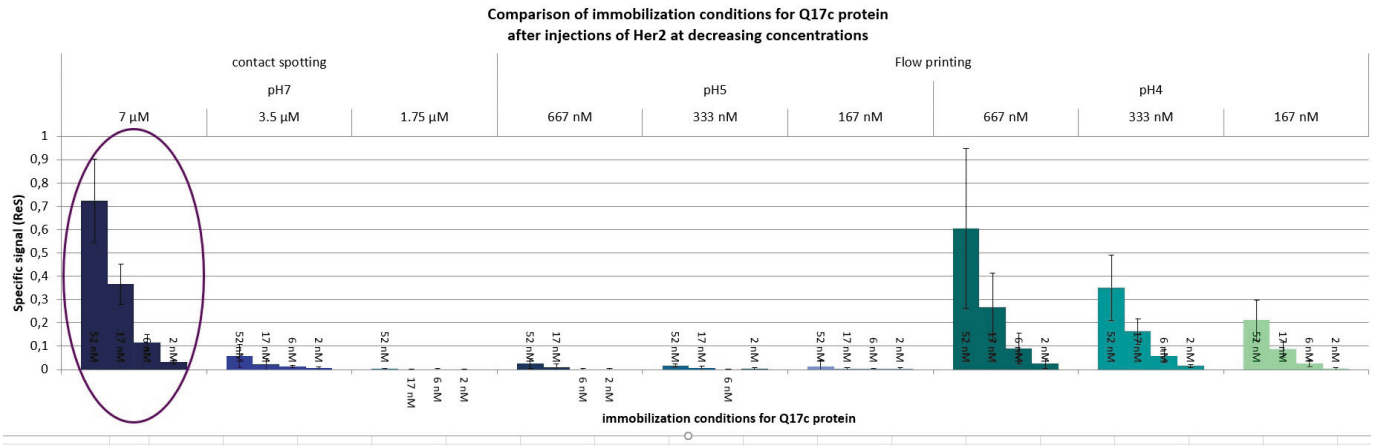

Figure 4: Specific responses retained for Q17c protein immobilised using two different spotting systems, at three different pH levels and at six different concentrations after the injections of HER2 protein at 2, 6, 17 and 52 nM.

immobilisation of different molecules and/or testing of different immobilisation conditions on a single biochip.

Specific HER2 protein binding responses retained on the spots of Q17c are represented in Figure 4. HER2 protein was injected at four different concentrations following a three-fold dilution series: 2, 6, 17 and 52 nM. For each injected concentration, the specific binding responses were measured during the dissociation phase of HER2 protein injections at the same time point.

Values are reference-subtracted and spot-averaged.

Specific binding responses were observed while injecting HER2 protein at different concentrations for Q17c immobilised at:

- 7 µM, in 10 mM PBS pH7.4 using contact spotting and
- pH4.0, whatever the concentration, using flow printing.

No binding was observed for Q17c immobilised in the other conditions.

Optimal binding responses of HER2 protein were obtained for Q17c immobilised at 7 µM in 10 mM PBS pH7.4 using contact spotting system.

In this study, similar specificity and affinity as conventional antibodies were also observed for single-domain antibodies.

We demonstrated here how optimisation of immobilisation conditions can be fast. These optimal conditions can then be applied to design a chip with multiple different VHH molecules.

Indeed, VHH molecules have key advantages that perfectly fit to biosensors to which SPRi technology belongs.

Conclusion

Antibody production companies are always looking for new technologies that allow them to accelerate the validation of their production and processes. They are looking for a solution that has the fewest steps, a solution that uses the least volume of reagents and a solution that provides a real-time response. Antibodies are commonly studied through the gold standard technology. By experimenting with the surface plasmon resonance imaging technology via three applications presented in this article, we highlight the key advantages of this platform: sensitivity for the detection of small molecules, screening potential and rapid experiment optimisation thanks to the label-free, real-time and multiplex capacities.

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Dr Chiraz Frydman is currently Global Senior Product Manager for SPRi and life science instruments. She has an engineering diploma in biology and a PhD in enzymatic engineering, bioconversion and microbiology. She has more than 20 years' expertise in biophotonics and more than 10 years' experience in optical instrumentation for molecular interaction analysis. For more than 10 years, she has been involved in more than 10 national and European projects.

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