How Making Screening is Easy Thanks to SPRimaging – A Label-free Molecular Interaction Analysis Technology

HORIBA Scientific's unique surface plasmon resonance imaging (SPRi) instrument enables a large number of different label-free molecular interactions to be monitored simultaneously and in parallel in a single experiment on a single biochip. The high throughput of the system provides superior capacity in comparison with channel-based SPR systems, allowing fast matrix experiments and analyses to be undertaken. Performing speed screening at a high throughput, XelPleX is the perfect automated system for this need. It allows measurement of hundreds of simultaneous antibody-antigen interactions in a mean time. Moreover, biologic molecules can be analysed in pure solution or in complex media such as cell supernatants, hybridoma solution, cell extracts...

Multiplexing helps to rank and screen molecules such as antibodies, Affimers and aptamers according to affinity or kinetic rate parameters easily and rapidly. High throughput screening is the best way to accelerate the validation and selection of molecules while reducing both time and costs.

In this article, we will highlight the example of Affimer screening, which is a perfect illustration of the power of SPR imaging. Affimers are next-generation affinity scaffolds with potential applications as novel biotherapeutics and renewable research and diagnostic tools. They are patented molecules produced by Avacta.

A few redundant terms will be used in this document, like ligand, analyte and biochip. The biochip consists in a high index glass prism coated with a thin gold layer. The metal is necessary to enable the effect of surface plasmon resonance. Other metals such as aluminum and silver can be used, but gold gives best results when we work with a liquid medium. The ligand is the molecule immobilised on the biochip surface and the analyte is the molecule (or solution) we inject in order to measure the binding (Figure 1).

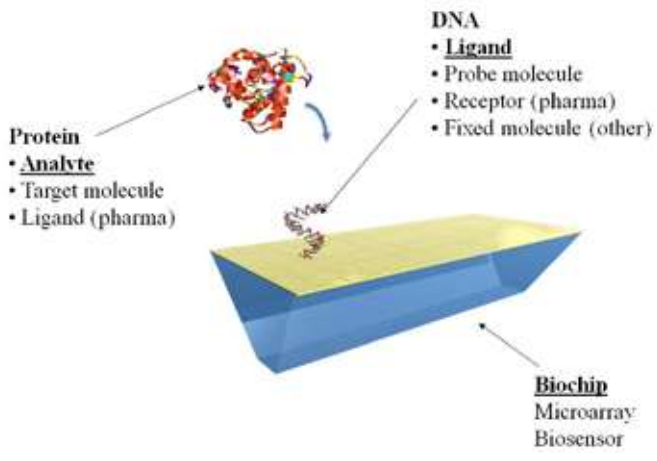

Figure 1: Ligand/Analyte

What is SPRimaging

Surface plasmon resonance imaging (SPRi) is a label-free optical detection technique used to monitor and analyse biomolecular interactions in real time. The imaging capability enables users to visualise the entire working area and work in a multiplex format.

Multiplexing means that different types of ligands can be immobilised on a single SPRi-Biochip. It also allows the study of many parameters at the same time (concentration, immobilisation pH, etc.), making it possible to compare, rank and select molecules easily and in the same environment.

Our technology measures modifications of the refractive index at the surface of the SPRi-Biochip, which can be correlated to mass variations. It can be used to detect interacting molecules in real time, to determine the analyte concentration, and the affinity of the interaction.

SPRi allows the full characterisation of biomolecular interactions (specificity, kinetics and affinity) which in turn can give information on a sample solution (quantity of molecules bound to the ligands, concentration of molecules).

A vast library of samples can be analysed. They include proteins, peptides, nucleic acids, carbohydrates, bacteria, cells, polymers, organic small molecules, and more.

SPRi is used to monitor changes of the refractive index occurring at the surface of the SPRi-Biochip. A binding event, or mass accumulation, will induce a change of refractive index and a shift of the position of the resonance angle. SPRi follows the variations of reflectivity occurring at a fixed angle (working angle) versus time.

The principle is described in the diagram of Figure 2:

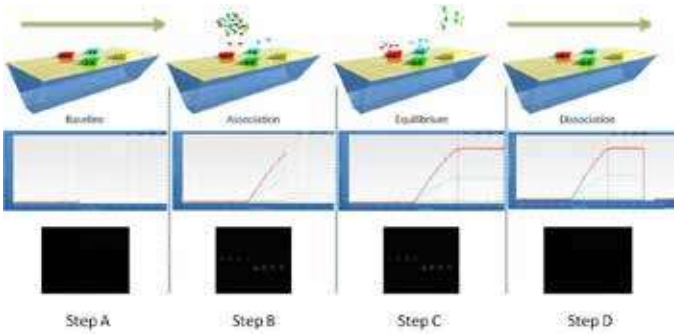

Figure 2: Monitoring of molecular interactions by SPRi

- Step A: The ligands are immobilised in an array format on the functionalised SPRi-Biochip surface. No interaction occurs as there is no modification of the SPRi signal.
- Step B: When the sample solution is injected in the flow cell, molecular binding can occur. This induces a shift

of the plasmon curves and an increase of reflectivity. The kinetic curves (sensorgrams) show the variations of reflectivity versus time (association phase). The process can also be monitored on the SPRi difference image. White spots correspond to interacting areas of the SPRi-Biochip.

- Step C: When the sample solution leaves the flow cell, the ligand-analyte complexes dissociate. This induces a shift of the plasmon curves and a decrease of reflectivity. The kinetic curves show the variations of reflectivity versus time (dissociation phase). The process can also be monitored on the SPRi difference image as interacting spots become darker.

- Step D: When all the ligand-analyte complexes are fully dissociated (sometimes using a regeneration solution), the plasmon curves and the kinetic curves return to the initial state. The SPRi difference image is black again.

Brief History of the Technique[1]

In 1902, Wood, observing the spectrum of a continuous source of white light using a diffraction grating in reflection, noticed thin dark bands in the diffracted spectrum[2]. Theoretical analyses undertaken by Fano[3] in 1941 led to the conclusion that these anomalies were associated with surface waves (surface plasmon) supported by the network. It was in 1968 that Otto[4] showed that these surface waves can be excited by using attenuated total reflection. In the same year, Kretschmann and Raether[5] obtained the same results from a different configuration of the attenuated total reflection method. Following this work, the interest in surface plasmons has increased considerably, in particular for characterising thin films and for studying processes taking place on metal interfaces. Marking a turning point in surface plasmon applications, Nylander and Liedberg, for the first time, in 1983, exploited the Kretschmann configuration for gas and biomolecules[6] detection. The different possibility of exploitations in this field and the need for more and more robust and reliable devices allowing the understanding of biomolecular phenomena gave birth to companies specialising in the sale of SPR devices.

In the 1980s, surface plasmon resonance (SPR) and related techniques exploiting evanescent waves were applied to the interrogation of thin films, as well as biological and chemical interactions. These techniques allow the user to study the interaction between immobilised ligands and analytes in solution, in real time and without labelling of the analyte. By observing binding rates and binding levels, there are different ways to provide information on the specificity, kinetics and affinity of the interaction, or the concentration of the analyte.

Label-free Screening of Affimers

Affimers are small single domain proteins of around 98 amino acids (aa), with molecular weight of 10 kDa (without inserts), which are derived from the cystatin family protein fold (Fig. 3). Interactions are generated through insertion of random peptide sequences in loops 2 and 4. The main benefits of Affimers are their high specificity for targets and the stability of the molecule compared to antibodies. Furthermore, the rapidity of development and the ease of manufacturing, which enable production of custom reagents in weeks instead of months, provide clear advantages for this technology.

Affimer technology has a number of essential advantages over other affinity technologies, such as antibodies and aptamers.

- **Rapid development** – Save time, get custom reagents in 12–14 weeks
- **Engineered specificity** – Design your screening process to select desired binding properties
- **Flexible fictionalisation** – Easily format Affimer sequences to maximise performance in multiple applications and assays
- **Ease of manufacturing** – Consistent and cost-effective production, using simple bioprocesses
- **Not limited by the immune system** – Enable difficult targets that would be challenging for antibody technologies

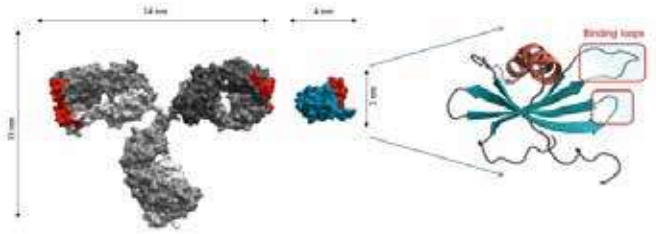

Figure 3: Affimer alternative to antibodies as affinity reagents

During the production process, hundreds of Affimers specifically selected by phage display against target molecule are generated. Only the best candidates are selected at the end of the bioprocess. Screening Affimers is one of the critical steps of the entire process (Fig. 4). Thus, a rapid binding screening phase was inserted in the entire process. This phase is based on the surface plasmon resonance imaging technology.

Figure 4: Affimer discovery process

Preliminary experiments were carried out in order to optimise experimental conditions. The surface plasmon resonance imaging (SPRi) technology of the XelPleX instrument was used as a tool to study the binding of target antibodies to candidate Affimers (Affimers were used as ligands and the antibodies were used as analytes injected during the experiment).

Affimer clones, selected by phage display for their ability to bind specifically to antibodies, were immobilised in different buffers and at different concentrations on a single XelPleX SPRi-Biochip. The target antibodies were injected to monitor the binding in real time.

As shown here, more than 50 Affimers were screened under different experimental conditions. The best candidates and the best conditions were selected based on the binding affinity. Working on a multiplex format leads to the generation of much data. In order to keep the data analysis simple, a 'cloud

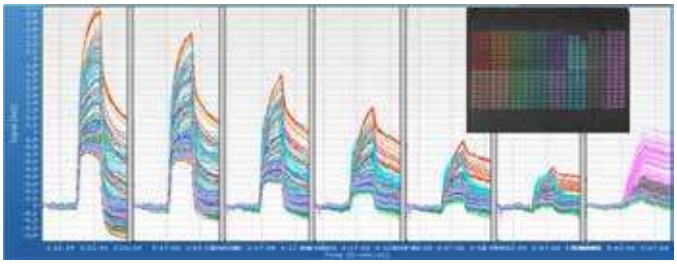

Figure 5: Kinetic curves of 480 interactions and imaging of the SPRi-Biochip surface

display' option was developed in the software. The cloud tab is a powerful tool, allowing rapid translation of all the curves into a single plot containing a cloud of data points. The Y axis represents the relative dissociation of the Affimer/antibody interaction over a defined time period during the dissociation phase, expressed as a percentage, and the X axis represents the absolute response value obtained at the end of the association phase expressed in ReS (resonance shift).

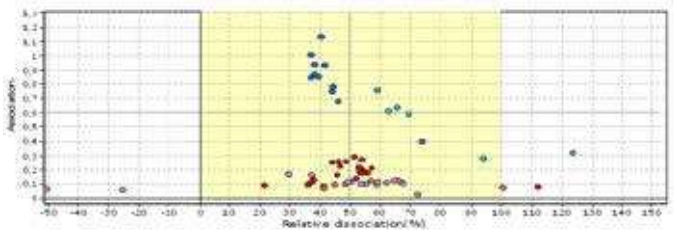

Figure 6: Cloud analysis

From the cloud tab, we can rapidly select and focus on the Affimer groups of interest and more deeply analyse these data in order to determine the binding parameters (Fig. 7).

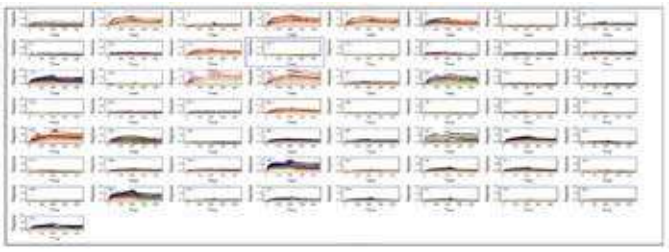

Figure 7: Binding curves for different Affimers

Conclusion

The multiplexing configuration of the XelPleX allowed testing different immobilisation conditions (buffer preparations, pH, concentration ...) for the Affimer clones. It showed the high benefit of using SPRimaging to save time and money and also to have access to an off rate constant. Indeed, this study demonstrated the ability to study 50 samples simultaneously on a single chip, which led to:

- high time efficiency
- comparability between samples in the same environment
- repeatability of the results
- relatively low cost of consumables for high throughput SPR

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Dr Geoff Platt

Dr. Platt leads a team of scientists developing novel applications for Avacta's Affimer technology. Affimer reagents are engineered binding proteins that display utility in a range of biotechnological applications. Geoff has previously established new applications for analytical instruments at Avacta Analytical and Farfield Group (UK). He obtained a PhD in chemistry from the University of Nottingham and spent a number of years at the University of Leeds studying protein structure, stability and folding kinetics, as well as underlying mechanisms of protein aggregation.

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