Super Screening

By using new imaging technology, it is now possible to detect and quantify a biomarker in different supernatants with only a single injection. Analysing crude samples at a high-throughput has thus become much more realisable

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To fully understand the details of a biological mechanism at the molecular level, it is important to characterise the biomolecular interaction in a relevant environment without modifying the samples or applying purification procedures.

Enabling this is the advent of a robust and easy-to-use fluidic system, and the injection of complex samples without dilution – such as serum, plasma, cell supernatants or even lysates – is no longer an issue for diagnostic research or drug screening. Moreover, crude samples can also be immobilised on the sensor chip for a rapid and high-throughput quantification that can be utilised in pharmaceutical R&D. The immobilised biological molecules may be quantified in different crude supernatants with only a single injection of the target.

The following discussion examines a biomarker – which we will call 'Protein Y' (PY) – which is a component of the HIV structure. This protein is commonly employed as an AIDS diagnosis tool in combination with other immunological tests. Until recently, enzyme-linked immunosorbent assay (ELISA) has been the standard method for PY-based diagnoses. Nevertheless, this method shows some limitations in terms of rapidity and high-throughput,

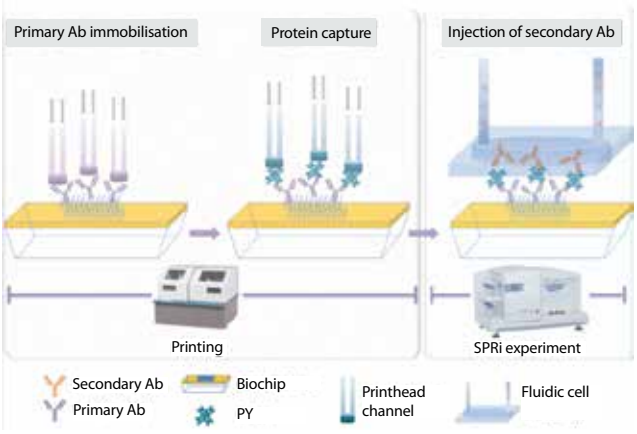


Figure 1: Assay set-up

	A	B	C	D
1	Supernatant 1	Supernatant 1	Supernatant 1	Supernatant 1
2	Supernatant 2	Supernatant 2	Supernatant 2	Supernatant 2
3	Supernatant 3	Supernatant 3	Supernatant 3	Supernatant 3
4	Supernatant 4	Supernatant 4	Supernatant 4	Supernatant 4
5	Supernatant 5	Supernatant 5	Supernatant 5	Supernatant 5
6	NC	NC	NC	NC
7	PC	PY C5	PY C3	PY C1
8	PC	PY C5	PY C3	PY C1
9	PC	PY C5	PY C3	PY C1
10	PY C7	PY C6	PY C4	PY C2
11	PY C7	PY C6	PY C4	PY C2
12	PY C7	PY C6	PY C4	PY C2

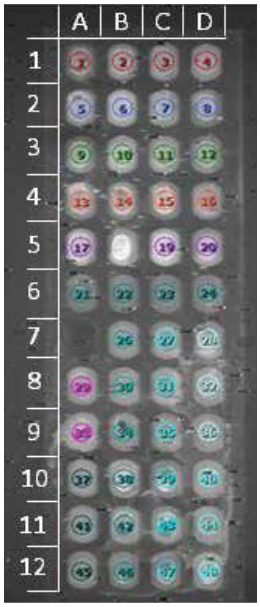


Figure 2: Printing pattern (A) and image of the biochip (B)
(Masks superimposed on B). NC: negative control, PC: positive control. PY C1: PY at 10 ng/mL, PY C2: PY at 40 ng/mL, PY C3: PY at 120 ng/mL, PY C4: PY at 370 ng/mL, PY C5: PY at 1.1µg/mL, PY C6: PY at 3.3 µg/mL, PY C7: PY at 10µg/mL

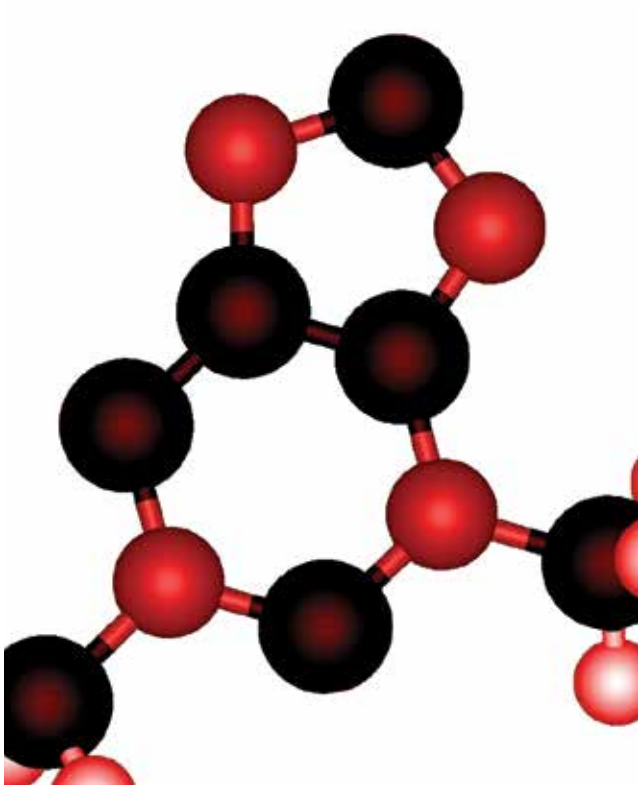
which are increasingly required in pharma R&D. Now, however, there is a good alternative to the use of ELISA in determining the protein concentration. Unlike ELISA – which needs labelling and is time-consuming – surface plasmon resonance imaging (SPRi) is label-free and takes only a few minutes to screen a huge panel of samples.

Materials and Methods

During the printing phase, the use of the microfluidic immobilisation method generates higher spot homogeneity and immobilisation levels. SPRi allows the monitoring of the interaction in a label-free way and in multiplex formats (see Figure 1, page 38).

Step 1: Primary Antibodies Immobilisation
Primary antibodies (Abs) directed against PY were immobilised. All capture Abs were prepared at 1µM in 10mM sodium acetate at pH5 and immobilised on the activated surface of a biochip.

Step 2: Protein Capture
PY was captured at 10ng/mL, 40ng/mL, 120ng/mL, 370ng/mL, 1.1µg/mL, 3.33µg/mL and 10µg/mL. This range was chosen to cover the concentrations of the tested supernatants, and all of them were used without dilution. A negative control (anti-Ovalbumin immunoglobulin G (IgG)) and a positive control (PY spiked in crude medium) were also immobilised. Both purified PY and anti-Ovalbumin IgG were diluted in 10mM phosphate buffered saline (PBS) pH7.4. After the immobilisation procedure, the biochip was blocked using 1M ethanolamine. The printing pattern and the image of the printed biochip can be seen in Figure 2.



SPRi Experiment
The printed biochip was then loaded into an instrument for the analysis of biomolecular interactions, which were monitored using software designed specifically for examining high-throughput of molecular interaction assays. The running buffer stood at 10mM PBS pH7.4 and the working temperature was set

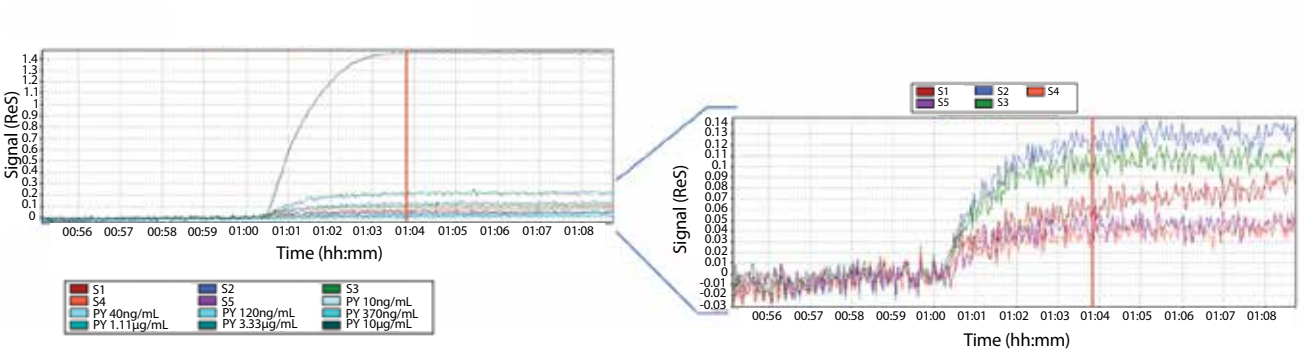
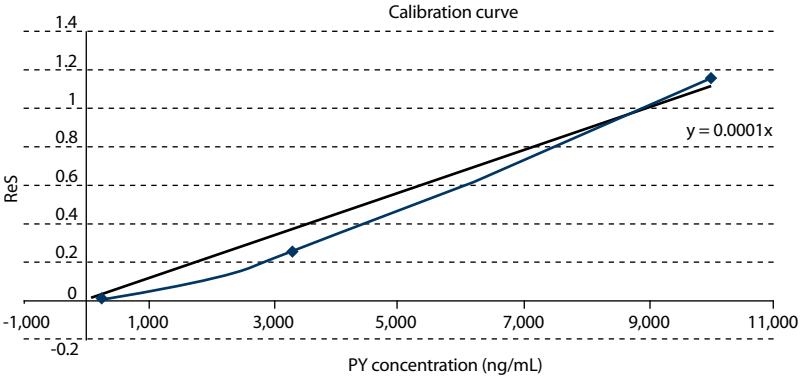


Figure 3: Averaged and reference-subtracted kinetics curves (A) and zoom on the curves corresponding to the five supernatants (B) after injection of the secondary Ab at 100nM



Sample	PY concentration in µg/mL
Supernatant 1	0.73
Supernatant 2	1.36
Supernatant 3	1.09
Supernatant 4	0.44
Supernatant 5	0.42

Figure 4: Calibration curve and PY concentrations determined in the supernatants

to 25°C. Then, 200µL of the secondary Ab were injected into the fluidic system at a flow rate of 50µL per minute.

After a blank injection (injection of the buffer), the secondary Ab was injected at 100nM. Figure 3 reveals the kinetic curves obtained for the purified PY captured at known increasing concentrations and for the supernatants (A) and PY taken from the five tested supernatants (B). It also shows that PY was detected in all supernatants tested.

A calibration curve was drawn based on the responses obtained for each concentration of purified PY immobilised (see Figure 4). The curve was used to evaluate the concentration of PY in the different supernatants in a single biochip and with a single injection.

Conclusion

The multiplex feature and the flexible fluidic system allow protein quantification in several crude samples simultaneously, facilitating both time and cost savings. Through a single injection, determining the concentration of PY in five different supernatants – by using a calibration curve that is established on the same biochip – is now a reality.

This application demonstrates that SPRi is a powerful tool for rapid and high-throughput quantification processes. On a single biochip, 14 different conditions were tested and 48 sensorgrams were generated in under 15 minutes.

About the authors

Fatima-Ezzahra Hibti joined Genoptic – which later became HORIBA Scientific – as Application Engineer in 2009 after graduating from the Université de Reims Champagne-Ardenne, France, with a Master's degree in Biotechnology and Therapeutic Strategies. For more than seven years, she was involved in various national and European projects and is now in charge of customer feasibility experiments. Fatima-Ezzahra is currently working with the life sciences team to develop the SPRi platform, as well as new applications for it in collaboration with the SPRi team and external partners.
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