Protein detection in cell culture media: P4SPR vs large SPR device

Abstract

Here, we demonstrated how Portable, 4-channel SPR (P4SPR) can directly detect a protein in complex media from bioproduction. The model molecule is PD-L1 and the performances of the P4SPR are compared with a larger SPR device.

Introduction

Although label-free, real-time sensing SPR is crucial in bioproduction, its use is often limited by the equipment cost and complexity of operation. To be practical, SPR must be more accessible. Affinité's P4SPR offers ultra-compact, convenient SPR at the benchtop with no compromise on data quality of protein interaction characterization. To take full advantage of having SPR at the bench, the P4SPR uses static measurement. The sample is injected directly onto the sensing area and remains in contact with the surface for minutes without any flow. Protein can be quantified, and affinities estimated with this method. Table 1 outlines key practical considerations when using SPR. In addition to the faster training time, shorter run time and easy maintenance, experimental protocols developed on P4SPR are directly translatable to larger SPR systems.

Practical considerations for SPR	P4SPR	Large SPR
Training time	< 1h	Days
Run time	Minutes	Hours
Maintenance	Accessible fluidic	Inaccessible fluidic
Sample flow	None (static)	> 5 uL/min
Limit of quantification for antibodies	high pg/mL	mid pg/mL
Volume (uL)	200	<100
Accessibility	Benchtop	Centralized lab

PD-L1, or programmed death-ligand 1, is a protein that plays a crucial role in the regulation of the immune system. It is found on the surface of certain cells, including some cancer cells. PD-L1 interacts with a receptor called PD-1 (programmed cell death protein 1) on immune cells, such as T cells. Given its importance in cancer immunotherapy, the PD-1/PD-L1 interaction was selected as model. PD-L1 was produced in mammalian cells.

Assay design

The carboxyl surfaces were first activated by EDC/NHS. The PD-L1 and control antibody were then immobilized at similar levels. The surfaces were deactivated with ethanolamine and finally primed with a regeneration buffer consisting of glycine HCl and washed with running buffer. Figure 1 shows the immobilization, where two control surfaces were prepared per sensor: one that had undergone the activation and deactivation process with no ligand (Background) and another with a control ligand immobilized to the surface, the detector antibody surface was duplicated.

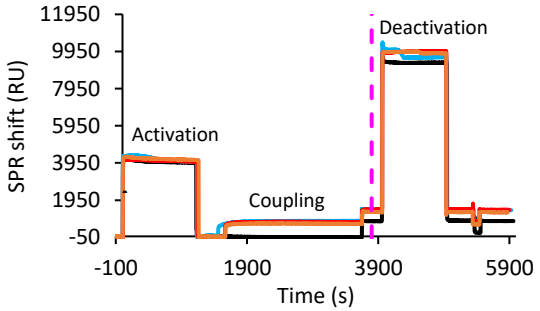


Figure 1: Immobilization of control (blue) and detector antibodies (red, orange) at values of 471RU, 468RU and 605RU respectively.

A proprietary surface chemistry demonstrated to reduce non-specific binding of complex media was used. Two P4SPR sensors were prepared following the described protocol above:

activation, immobilization of Abs and passivation. Two sensors with the same surface chemistry were then prepared using the same protocol in a different SPR device.

PD-L1 showed binding on all 4 sensor surfaces, Figure 2 shows binding of human PD-L1 protein to rabbit monoclonal Anti-PD-L1 antibody for one concentration. The signal intensity after rinsing with running buffer is plotted against PD-L1 concentration in Figure 3 showing a sigmoidal response curve and a dynamic range of 20nM to 400nM. PD-L1 was also added to cell culture supernatant where detection was possible on all 4 sensors.

The SPR shift in RU and Reflectivity (%) was measured at equilibrium for each sample solution and the background channel signal was subtracted from the detector antibody signal.

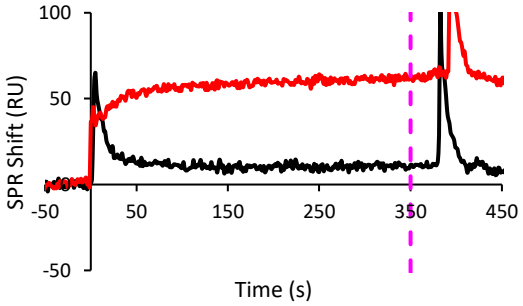


Figure 2: 1ug/ml (40nM) PD-L1 injection in buffer on detector antibody (red, top) and background (black, bottom), the SPR shift was measured at equilibrium at 350 seconds.

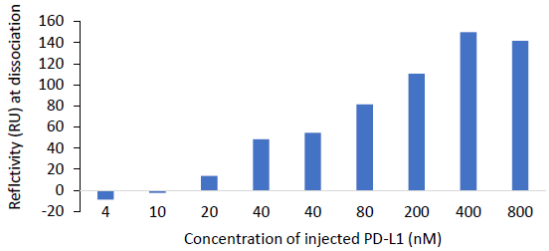


Figure 3: Detection of PD-L1 sensitivity after background subtraction

Comparing P4SPR to large SPR device

To compare the analytical performances of the proprietary surface chemistry, a protocol was first developed on the large SPR device (flow based) and transferred to the P4SPR (static based). The culture media was used as blank, then the media containing PD-L1 was injected across the sensor and compared against a known PD-L1 spiked in media.

Figure 4 illustrates the PD-L1 detection protocol performed on both the P4SPR (top) and a larger SPR device (bottom). PD-L1 detection directly in undiluted culture media was achievable on both devices. However, differences were observed in the culture media responses. A non-specific response of about 10 RU was observed on the P4SPR, while a negligible response was noted on the larger SPR device. The main difference between these devices is the mode of sample introduction. The larger SPR device operates under constant flow conditions, which washes away weak interactions. In contrast, the P4SPR operates under static conditions, allowing weak interactions to occur on the surface. While this non-specific interaction on the P4SPR can be reduced with further assay optimization, the high PD-L1 signal in cell culture media suggests that the level of detection is adequate for screening purposes. Another noticeable difference is the reference signal. However, a comprehensive analysis of this difference would necessitate further testing due to varying buffer conditions between the two platforms.

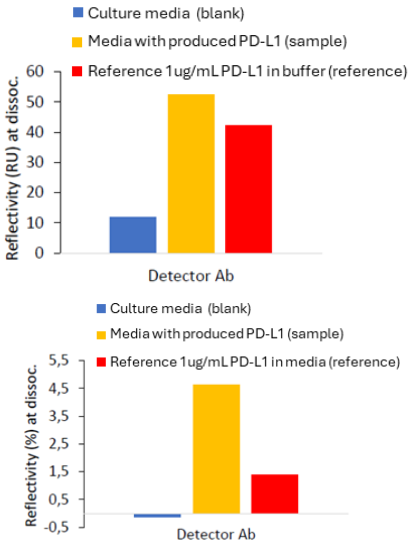


Figure 4: PD-L1 detection assay (RU) on P4SPR (top) and large SPR device (bottom)

Binding consistency of P4SPR

The binding response of a 40nM PD-L1 sample solution in running buffer was evaluated between 2 P4SPR sensors where the anti-PD-L1 antibody was immobilized following the same procedure. The averages are presented in Figure 5. Sensor 1 and sensor 2 had an average signal response of 49RU (n=6) and 48RU (n=4) respectively.

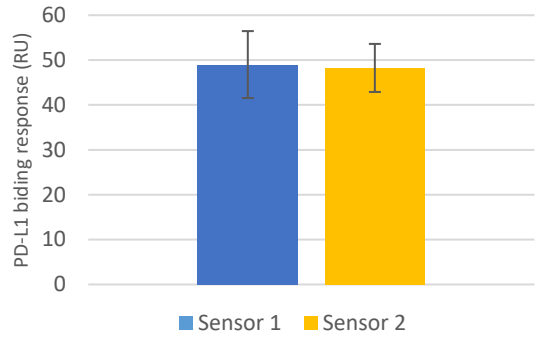


Figure 5: Average binding response to PD-L1 in RU evaluated with P4SPR across 2 sensors.

The binding response of a 40nM PD-L1 sample solution in running buffer was of the same sensor were then compared over 2 days. The average binding responses are shown in Figure 6, values for day 1 and day 2 were 51RU (n=6) and 44RU (n=3) respectively representing a signal decrease of only 14%.

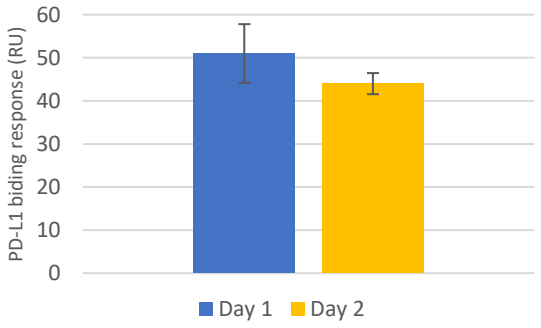


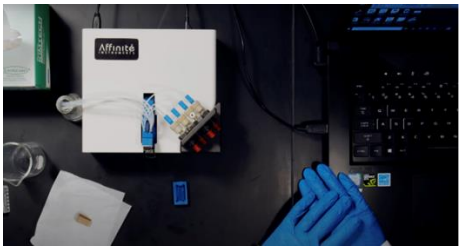
Figure 6: Average binding response to PD-L1 in RU evaluated with P4SPR over 2 days across 2 sensors.

Conclusion

In a bioproduction context, real-time SPR sensing was achieved, showcasing the practicality and user-friendly nature of the instrument for a first-time user. The ultra-compact P4SPR device not only ensures ease of use but also delivers high-quality data comparable to larger SPR devices for the characterization of protein interactions.

The Anti-PD-L1 antibody was immobilized on 2 P4SPR sensors and a large SPR device sensor. PD-L1 binding was observed on all 4 sensors in their respective instruments in two different media, running buffer and complex media. PD-L1 binding levels were shown consistent across multiple measurements over 2 days.

The P4SPR advantage



Affinité instruments P4SPR is a modular, label-free multichannel system with great application versatility. Its accessibility and ease of use allow for rapid high quality SPR results in antibodies characterization. Its multichannel feature increases result precision and additionally, with the Affinité manufactured sensors, non-specific interactions are greatly reduced. This would be especially interesting for studying interactions in crude samples.

The wider application of the P4SPR to more complex systems have considerable potential to study a variety of antigen-antibody interactions and can be extended to other biomolecular interactions.

About Affinité Instruments

Established in 2015 as a spin-off of the Université de Montréal, Affinité instruments' foundation is built deep knowledge accrued on over a decade of research in SPR. The commercialization of promising innovations is spearheaded by a diverse leadership experienced in business, science and engineering.