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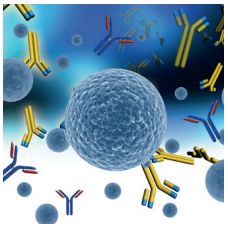
Surface Plasmon Resonance imaging

Simple injection VS. recycling Kinetic profile and affinity comparison



Technical Note
Biology
SPRi TN08

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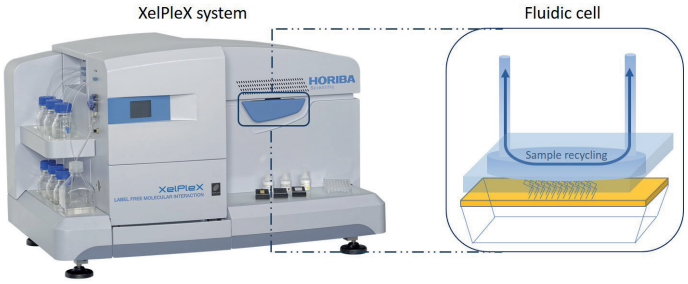
Working with crude samples can be challenged by two situations: First the molecule of interest is one among many others, and second it is present at a low concentration. The XelPleX system can overcome this limitation thanks to the recycling feature. The user can run the sample over the surface of the sensor chip several times, leading to a better capture of the molecules. Recycling the sample increases the contact time between the analyte injected and the ligand immobilized on the biochip surface. This technical note details how to use the software to set up the recycling feature and shows its relevance in the case of an affinity study.

Keywords: Sample recycling, affinity constants

Introduction

The XelPleX system is based on the Surface Plasmon Resonance Imaging technique that monitors molecular interactions in real time for kinetic characterization purposes. Thanks to its special fluidics, the XelPleX enables the injection of crude samples, like serum or cell lysates. One challenging parameter in such samples is the low amount of the molecule of interest. To overcome this limitation, the sample is recycled instead of being directed to the waste. Through the recycling feature, the XelPleX offers the possibility to increase the contact time between the immobilized ligand and the injected analyte without consuming more samples.



In this case, the question that can be asked is: Are the kinetic constants the same for recycling experiments and a simple injection? This technical note highlights the comparison of the extracted kinetic constants through a protein/antibody interaction model.

Software set-up

The parameters of each injection can be defined. In the “Injection definition” window, double click on the injection you want to edit. The following window will display as shown in Figure 1. Select the number of recycling cycles to be performed. Click on “Apply” to validate.

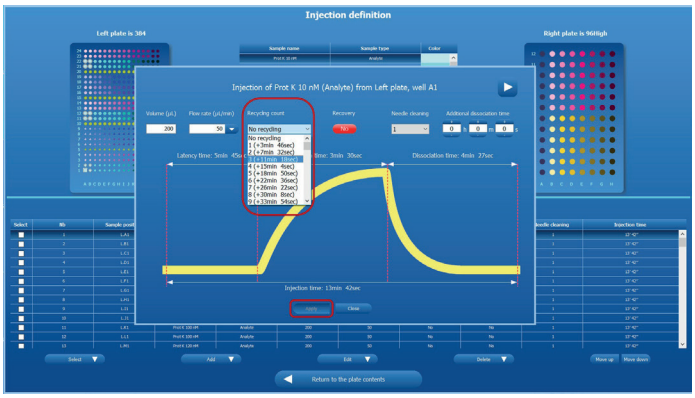


Figure 1: Injection’s parameters

Each recycling cycle will add supplementary minutes to the association time. The added time will depend on the flow-rate and the injected volume.

Applicative example: Anti-Ovalbumin/Ovalbumin model

A SPRi-Biochip was spotted using different proteins at two concentrations, as shown in Figure 2.

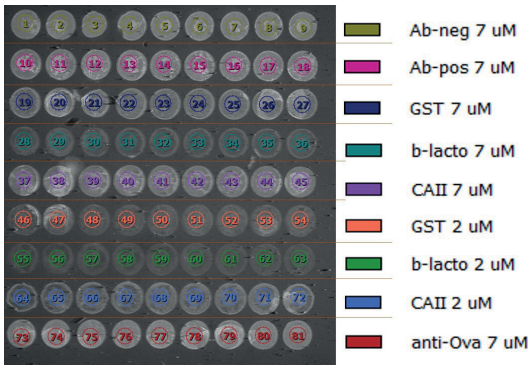


Figure 2: Spotting map

Ovalbumin was injected from 2.8nM to 678nM (2.8nM, 8.4nM, 25nM, 75nM, 226nM and 678nM). 100mM Glycine-HCl at pH2.0 was injected after each concentration to regenerate the surface.

Ovalbumin was first injected without recycling at 50µL/min. Then the same concentration range was injected with three recycling cycles at the same flow-rate. Figure 3 shows Ovalbumin injections with, and without recycling.

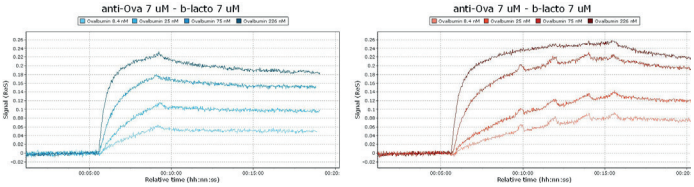


Figure 3: Overlaid and reference-subtracted kinetic curves after injection of Ovalbumin at increasing concentrations without (left) and with recycling (right)

Recycling enables the ability to target capture by increasing the contact time. The Figure 4 compares the signal for a simple injection after 4 minutes (blue) and for recycled sample after 15min (Orange). The signal increases by 44% for the lowest concentration and by 9% for the highest one. For the highest concentration, we observe a minimal improvement, and this is predominantly due to reaching the level of saturation.

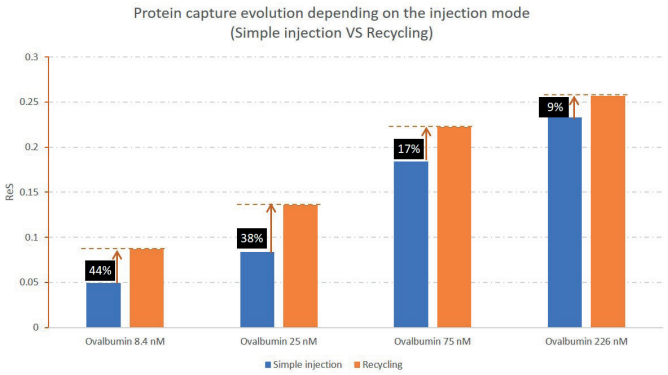


Figure 4: Overlaid and reference-subtracted kinetic curves after injection of Ovalbumin at increasing concentrations without (left) and with recycling (right)

To evaluate the kinetic constants, the kinetic curves were fitted using Ez-Fit software (Figure 5). The calculated KD is the same, regardless of type of injections (with or without recycling).

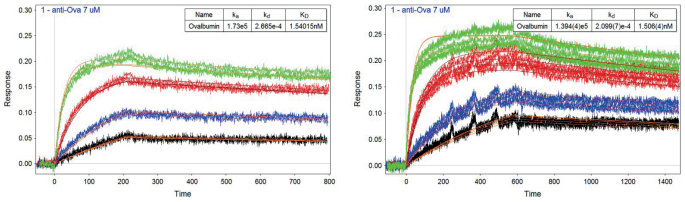


Figure 5: Fitted kinetic curves corresponding to injection of Ovalbumin at increasing concentrations without (left), and with recycling (right)

Conclusion

The XelPleX system offers several tools that meet different applications needs. In this technical note, we focus on the recycling feature that enables maximization of the capture of the analyte by increasing the contact time. Through the anti-Ovalbumin/Ovalbumin-based model, we demonstrate that the affinity constants remain the same for both injection modes: Simple, and with recycling cycles. The recycling feature can then be used without worrying about its impact on the affinity constants.

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