



A Single-Injection Step for Affinity Determination Using Surface Plasmon Resonance imaging

Surface Plasmon Resonance imaging (SPRi) enables a large number of different molecular interactions to be monitored simultaneously and in parallel in a single experiment. Throughput presents superior capacity in comparison with channel-based SPR systems, allowing fast matrix experiments and analyses to be undertaken.

In the classical format, the affinity constant measurement of a single ligand-analyte pair is possible thanks to the injection of dilution series of the analyte. In addition to the benefits of speed and throughput, the unique technology used within the HORIBA Scientific-GenOptics SPRi instruments is also suitable for affinity determination in a single injection step using a multiplex approach. The ligand is immobilized at different concentrations on the same surface, followed by a single concentration injection of the analyte. The advantage of running this process is to avoid regeneration steps, especially when:

- Interactions are too strong to be properly regenerated
- Ligands are sensitive to drastic regeneration solutions

In this technical note, the affinity of an antibody for a birch allergen is determined by performing both approaches.

Material and Method

Antibodies immobilization using SPRi-Arrayer™ on a CS SPRi-Biochip™

The CS SPRi-Biochip™ is made of a self-assembled monolayer of polyoxyde ethylene glycol activated using an EDC/NHS solution in preparation for amine coupling.

Figure 1 shows the pattern used for immobilizing molecules in an array format (spots). Antibodies directed against a recombinant allergen called rBet v 1 (a-rBet v 1) were prepared in 10 mM PBS pH 7.4 in a 2-fold dilution series format, and immobilized to the activated surface of the SPRi-Biochip™ using the SPRi-Arrayer™ by simply depositing drops. The highest spotting concentration was 6 µM and the lowest 47 nM. A reference molecule was also immobilized at the same concentrations for referencing purposes. Spots were immobilized in duplicates. After the immobilization procedure, the SPRi-Biochip™ was blocked using 1 M ethanolamine.

SPRi experimental details

The spotted SPRi-Biochip™ was then inserted into the SPRi-PlexII™ system from HORIBA Scientific - GenOptics. The running buffer was 10 mM PBS pH 7.4 and the working temperature was set to 25°C. Then, 190 µL of 36.6 nM, 16.6 nM and 5.8 nM rBet v 1 allergen were injected into the fluidic system at a flow rate of 50 µL/min. The surface was regenerated using 0.1 M glycine-HCl pH 2.0 after each allergen injection.

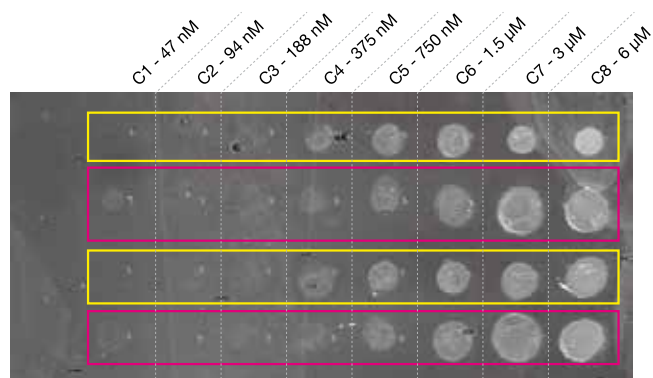


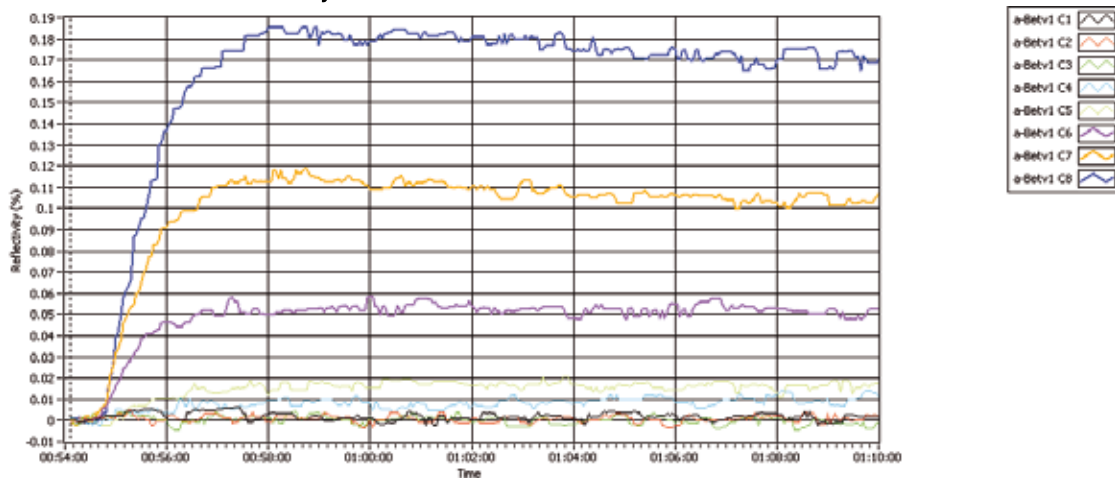
Figure 1: Image of the spotted SPRi-Biochip™. Spots framed in yellow correspond to anti-rBet v 1 antibody and spots framed in pink correspond to reference spots.

Results and discussion

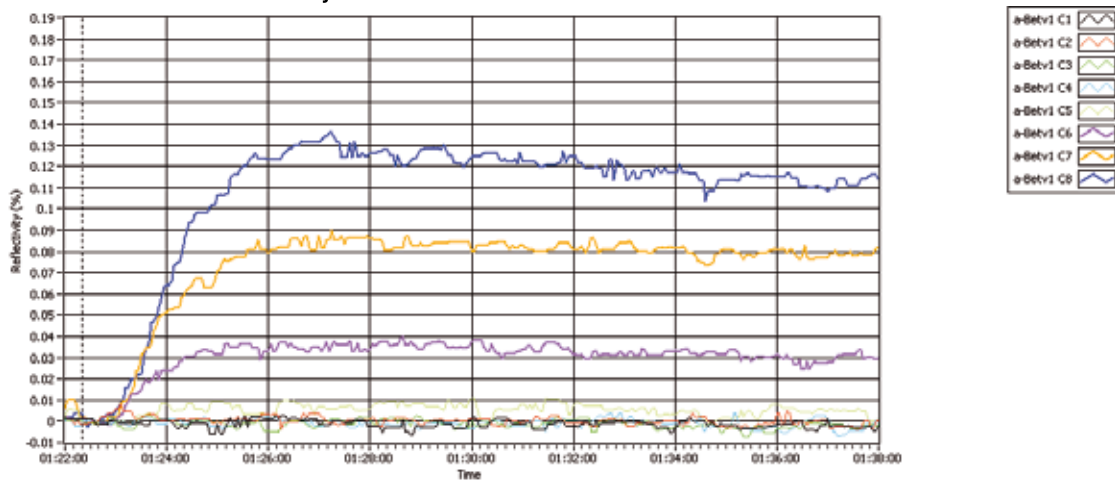
Figure 2 shows the average kinetics curves obtained for the binding of rBet v 1 allergen to anti-rBet v 1 antibody spots immobilized at 8 concentrations for three concentrations of the allergen (36.6 nM (A), 16.6 nM (B) and 5.8 nM (C)). The corresponding negative control curves are subtracted.

The interaction between rBet v 1 and anti-rBet v 1 is observed for an antibody immobilization concentration above 1.5 µM. Interaction responses increase with increasing immobilization concentration of ligands and with increasing injected concentration of analytes.

A - 36.6 nM rBet v 1 injected



B - 16.6 nM rBet v 1 injected



C - 5.8 nM rBet v 1 injected

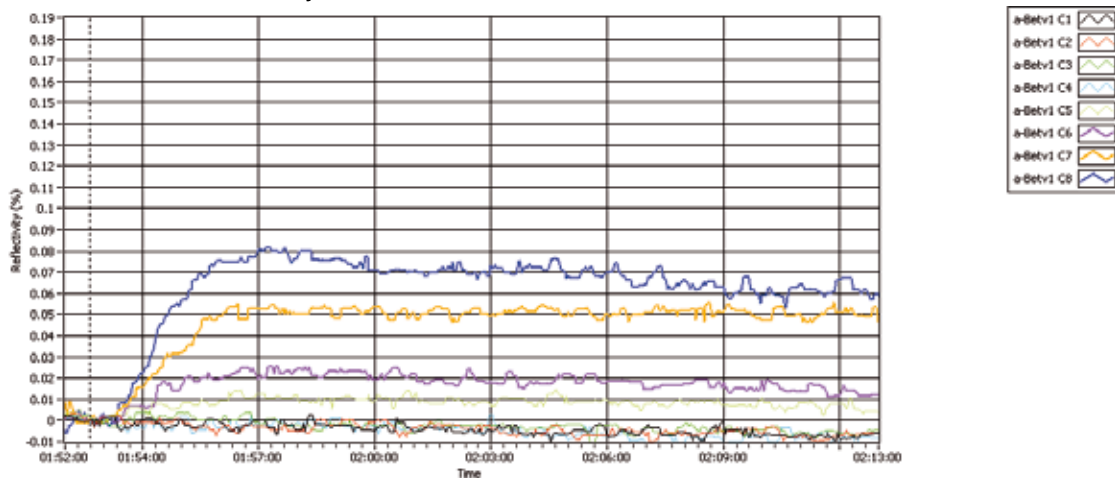


Figure 2: Real time kinetics curves (averaged and reference-subtracted curves) obtained after injections of rBet v 1 allergen at three different concentrations (A): 36.6 nM; (B): 16.6 nM and (C): 5.8 nM . a-Bet v1 antibodies are immobilized at different concentrations: C1 = 47 nM ; C2 = 94 nM ; C3 = 188 nM ; C4 = 375 nM ; C5 = 750 nM ; C6 = 1.5 μM ; C7 = 3 μM ; C8 = 6 μM.

First, the affinity was determined using the highest immobilization concentration of the antibody (6 μM) and the three injected concentrations of rBet v 1 allergen (5.8 nM, 16.6 nM and 36.6 nM) (top graph of Figure 3), as it is done in the classical approach.

Then, a second kinetic analysis was performed using the highest injected concentration of rBet v 1 (36.6 nM) and the three highest immobilization concentrations of the antibody which react (1.5 μM , 3 μM and 6 μM) (bottom graph of Figure 3).

To carry out such kinetic analysis, the ScrubberGen software was used. This software is suitable for processing multiplexed data intuitively. The SPRi signal obtained on reference spots were used for referencing. Then, the data was fitted globally using a local Rmax (i.e. maximum of reflectivity different for each curve) and a 1:1 interaction model.

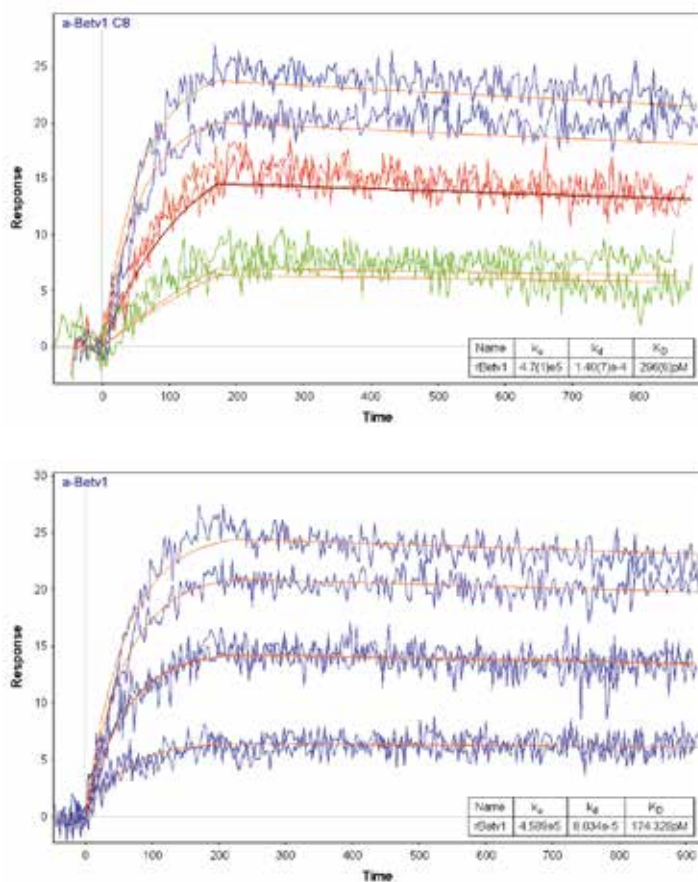


Figure 3: Kinetics analysis of anti-rBet v 1 antibody - rBet v 1 protein interactions (orange and black curves correspond to local fits using a 1:1 interaction model)

(top) 3 different injected concentrations of rBet v 1 (green curves: 5.8 nM, red curves: 16.6 nM and blue curves: 36.6 nM) for one immobilization concentration of anti-rBet v 1 (C8 = 6 μM)

(bottom) 3 different immobilization concentrations of anti-rBet v 1 (C6 = 1.5 μM , C7 = 3 μM and C8 = 6 μM) for one injected concentration of rBet v 1 (36.6 nM)

The kinetics parameters (association and dissociation rates) and the affinity of the antibody-antigen interactions determined according to the two methods are reported in Table 1.

	Kinetics Parameters		
	$k_a (\text{M}^{-1})$	$k_d (\text{s}^{-1})$	$K_D (\text{pM})$
«Classical» analysis	$4.7 \cdot 10^5$	$1.4 \cdot 10^{-4}$	296
«Single-injection» analysis	$4.6 \cdot 10^5$	$0.80 \cdot 10^{-5}$	174

Table 1: Kinetics parameters and affinities of the anti-rBet v 1 antibody - rBet v 1 protein interactions according to the two methods of kinetics analysis

The kinetics parameters and affinities determined are of the same order of magnitude with both methods (single ligand concentration and multiple analyte concentration injections versus multiple ligand concentrations and single analyte concentration injection).

Affinity constant determination is presented here for a single ligand immobilized on the SPRi-Biochip. However, using the multiplex approach such measurement is also possible for several ligands, with all of them immobilized on a unique surface.

Conclusion

This application note demonstrates the power of the multiplex approach for the determination of the kinetics parameters and affinities of biomolecular interactions using a single-injection step. Such method is well-adapted to the multiplexing capability of the HORIBA Scientific-GenOptics SPRi technology.

This original single-injection kinetics analysis has promising applications in fast antibody screening as it requires less amount of analyte solution. It will also solve applications for which a regeneration step is not permitted, because of the impossibility to force the dissociation of biomolecules, or because of the nature of the regeneration solutions used in SPR technology which may inactivate some immobilized biomolecules.

