Comparison of Single-Sequence and Multi-Sequence Kinetic Analysis of a Protein-Antibody Interaction on Alto

Summary

- Binding kinetics of Protein A with Human Immunoglobulin G (HIgG) were measured on Alto with both single-sequence and multi-sequence kinetics.
- K_D for this interaction was determined to be 1.93 nM and 3.39 nM with single-sequence and multi-sequence kinetics, respectively. This shows highly comparable results can be obtained for a given interaction with these different assay methods.

Figure 1. Alto Instrument

Overview

Alto is a high-throughput benchtop surface plasmon resonance (SPR) instrument. It provides high-quality binding kinetics and affinity data for a wide variety of molecular interactions *(Figure 1)*. As opposed to the more conventional fluid handling methods that utilize pumps and valves for sample handling and delivery to the SPR sensors, Alto uses digital microfluidics (DMF).

DMF is a liquid handling technology capable of accurately controlling and manipulating discrete nanoliter droplets with applied voltage giving the instrument wide flexibility over assay design.

Kinetic titration, also known as single-sequence kinetics (SSK) by Alto users, is a SPR analysis method wherein analytes are presented to the immobilized ligand sequentially in order of increasing concentration, with only a single dissociation and regeneration phase after all analyte concentrations have bound to the ligand on the sensor.

Alto is uniquely suited for SSK analysis by enabling exchanges of droplets on the sensor surface without the need for buffer flow between samples. In this application note, single and multi-sequence kinetics (MSK) for the same protein-antibody interaction were evaluated on Alto, showing the instrument's ability to facilitate both assay methods easily and accurately.

Material and Equipment

- Alto Instrument
- Alto CBX kinetics cartridge: Nicoya, SKU: KIN-CART-1-CBX
- Alto CBX coupling kit: Nicoya, SKU: ALTO-R-AMINE
- Nicoya Analysis Software
- Ligand: Protein A, Biovision, Cat # 6500B-10
- Analyte: Human Immunoglobulin G (HIgG), Sigma, Cat #I2511-10mg
- Running buffer: PBS-T (0.1% Tween 20), pH 7.4
- Regeneration: 10mM Glycine-HCl, pH1.5 + 0.1% Tween20

Procedure

1. Each experiment method was designed and created on the Nicoya User Portal.
2. Guided by the on-screen instructions in the control software, the cartridge was loaded into the instrument and samples/reagents were pipetted into appropriate wells. Steps 3-9 were performed automatically by the Alto instrument.
3. The carboxyl sensors were prepared using the cartridge clean activity with 10mM HCl for 60 s.
4. EDC and NHS solutions were mixed on-cartridge and used to activate the carboxyl surface for 300 s.
5. 40µg/ml Protein A in pH 5.0 sodium acetate buffer was immobilized on the sensor surface with an interaction time of 600 s on the response channel.
6. Ethanolamine was used to deactivate any unreacted carboxyl groups on the sensors for 300 s.
7. HIgG was diluted in the running buffer to a concentration of 900nM and loaded in the cartridge. Five concentrations of 3-fold serial dilutions were done on the cartridge.
8. For the MSK assay, samples were analyzed in order of low to high concentration for the following durations for each of the concentrations.
    - 120 s associations
    - 300 s dissociations
    - 60 s regenerations
9. For the SSK assay, associations for each analyte concentration were measured consecutively, followed by a single dissociation.
    - 120 s associations
    - 400 s dissociation
10. Binding kinetics were measured using Nicoya's analysis software with a one-to-one Langmuir fit model.

Results & Discussion

The ligand immobilization on the CBX sensors is shown in Figure 2, with over 2500 RUs of Protein A immobilization.

The binding curves and kinetic fit of the HIgG binding to the immobilized Protein A with a MSK assay are shown in Figure 3. HIgG 3-fold serial dilutions were automatically prepared on-cartridge prior to introduction on the sensor; for MSK each analyte sequence includes a regeneration with glycine-HCl pH 1.5 to return to baseline (Figure 4). Results were fit using a one-to-one binding model and local R_{max} to account for any potential surface modifications caused by the regeneration or analyte remaining bound to the ligand. The calculated kinetic constants are presented in Table 1.

The binding curves and kinetic fit for the SSK assay for the same interaction are presented in Figure 5. The same analyte concentrations and fit model as for the MSK assay were used with a global R_{max} and the calculated kinetic constants are presented in Table 1.

The data for each method was defined by subtracting the response of the reference channel from the active channel. Kinetic values in the low-nanomolar range were obtained for both assays. Goodness of fit was observed in the low relative Standard-Error-of-the-Mean (SEM) values and randomly distributed residual plots.

SSK analysis offers advantages over MSK; it shortens assay times and requires fewer regeneration sequences, thus reducing the risk of damaging the ligand when the sensor is used for multiple analysis sequences. SSK analysis is also useful for capture methods as it removes the need to recapture the ligand between analyte concentrations.

With traditional SPR instruments, there is a risk when using SSK due to only a single curve being generated for each analyte. If the data for any portion of the curve is compromised, it could render the results unusable for kinetic analysis. However, Alto's automated serial dilutions and digital microfluidic handling make the instrument less prone to user errors and data artifacts caused by air or contamination, as seen in traditional fluidic systems.

Both assay methods have their advantages and disadvantages and one may be preferable over the other for a given application. Alto's versatility makes this instrument suitable to run either method and generate high precision binding kinetics.

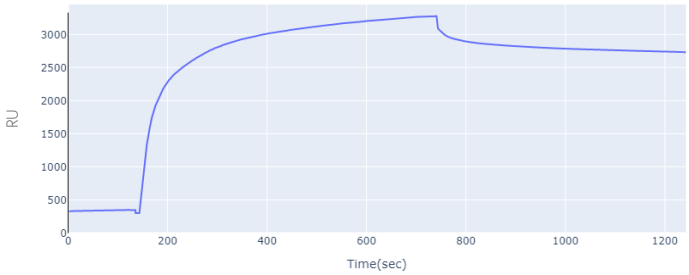

Figure 2: 40µg/ml Protein A immobilization on the CBX sensors.

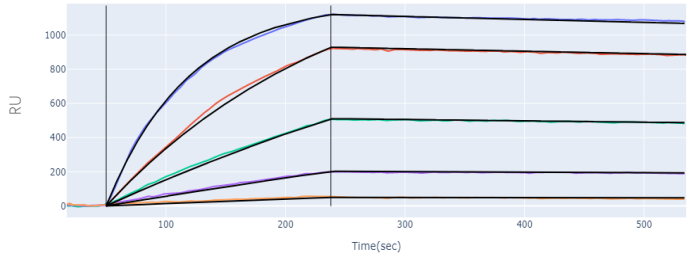

Figure 3: Multi-sequence binding of HIgG at 300 nM, 100 nM, 33 nM, 11 nM, and 3.7nM to the immobilized Protein A. The solid black lines represent the one-to-one kinetic model fits with local R_{max}.

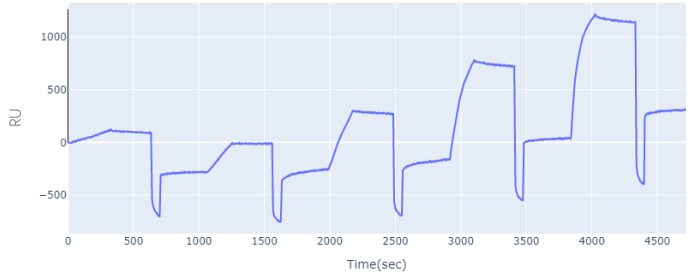

Figure 4: Full trace of the MSK assay showing the return to baseline after each regeneration. Full regeneration is not achieved for higher analyte concentrations. A local R_{max} was used in the kinetic fit to account for this.

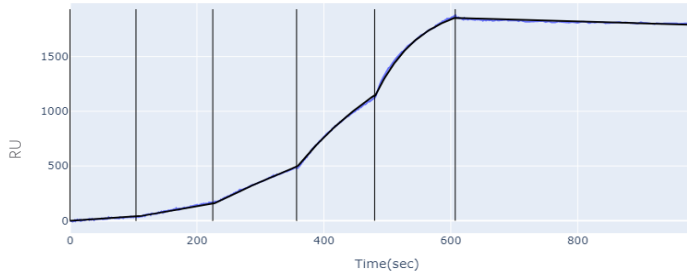

Figure 5: Single-sequence binding of HIgG at 300 nM, 100 nM, 33 nM, 11 nM, and 3.7 nM to the immobilized Protein A. The solid black line represents the one-to-one kinetic model fit with global R_{max}.

Table 1: Kinetics constants calculated for the SSK and MSK analysis for Protein A and HIgG using Nicoya's analysis software.

Kinetics Type	k_a [1/M*s]	k_d [1/s]	K_D [M]
SSK	4.67e4 (± 4.47e1)	9.04e-5 (± 1.10e-6)	1.93e-9 (± 2.53e-11)
MSK	4.75e4 (± 9.44e1)	1.60e-4 (± 3.25e-6)	3.39e-9 (± 6.95e-11)

Conclusions and Summary

This study demonstrates Alto's ability to analyze samples and measure binding kinetics with two different assay configurations. The kinetic binding constants obtained with the SSK method are consistent with those measured with the MSK assay for the same interaction and analyte concentrations. Alto's versatile fluid handling makes it a powerful tool for a variety of SPR assay designs and applications.