PROTEIN INTERACTION ANALYSIS USING ALTO

Digital Microfluidics Powered Surface Plasmon Resonance

Summary

- This application note details the first-ever integration of **digital microfluidics** and **surface plasmon resonance** to provide accurate affinity & kinetic analysis of a molecular interaction
- Alto determines the kinetics and affinity of a Protein A - IgG interaction, determining the following:
  •On-rate: 2.75 e^4 1/(M*s)
  •Off-rate: 8.15e-5 1/s
  •Affinity: 2.96 nM
- These compare well with the results determined using other techniques, but Alto requires 270X less sample and 50% less time to complete the analysis, demonstrating how Alto reduces the time and cost of drug discovery

Overview

Alto™ is a high-throughput benchtop surface plasmon resonance (SPR) instrument providing detailed binding kinetics and affinity data for a wide variety of molecular interactions *(see Figure 1)*. Alto uses digital microfluidics (DMF) instead of traditional pumps, valves and tubing for sample handling and delivery to the SPR sensors for label-free analysis. DMF is a liquid handling technology capable of accurately controlling and manipulating discrete nanodroplets with electricity. DMF-powered SPR significantly improves performance, data accuracy and consistency while enabling all sensors and fluidics to be contained in a single, disposable Alto Cartridge. This revolutionary technology only requires 2µl of sample for full kinetics, performs serial dilutions automatically on-chip, and delivers near instantaneous switching between buffer and sample.

Figure 1. Alto Instrument

Timely and accurate binding kinetics and affinity data is critical to the understanding of diseases and the discovery of drugs to treat them. Protein A and Human IgG is a well-known example of a protein-antibody interaction. Protein A is often used as a capture molecule for IgG antibody immobilization, providing a strong and reliable capture with a low dissociation rate. In this application note, Alto determines the affinity and kinetics between Protein A and IgG. This is the first ever demonstration of the measurement of binding kinetics and affinity using a DMF-powered SPR instrument.

Material and Equipment

- Alto™ Instrument
- Alto COOH Cartridge
- Ligand: Protein A (40µg/mL)
- Analyte: Human IgG (900nM IgG)
- Running Buffer: PBS-T (0.1% Tween 20), pH 7.4
- Regeneration Buffer: 10mM Glycine-HCl
- Nicoya Amine Coupling Kit

Procedure

1. Follow the software-guided Alto instrument setup procedure
2. When prompted, load the Carboxyl Cartridge into the Alto instrument
3. Pipette the following reagents into the Carboxyl Cartridge, following the on-screen instructions:

Table 1. Reagent type and volume used for Protein A - IgG experiment

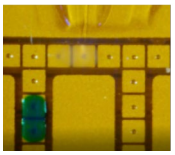
Reagent	Volume loaded (µL)
IgG	8.5
Protein A	35
10mM Gly-HCl	35
Blocking solution	35
0.1M EDC	8.5
0.1M NHS	8.5
PBS-T	105

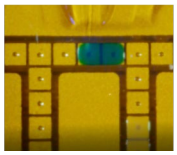
4. Once the cartridge is loaded and the experiment started, the following commands and procedures are executed automatically by Alto:
5. Prime the Carboxyl sensors with 10mM Glycine-HCl for 40 seconds
6. Activate the Carboxyl surface with EDC/NHS for 5 minutes
7. Immobilize Protein A in pH 5 at 40µg/ml for 10 minutes on Active Sensors followed by 5 minutes of washing in PBS-T
8. Block all sensors using blocking solution for 5 minutes to deactivate active Carboxyl groups
9. Prime the sensors with 10mM Glycine-HCl for 40 seconds
10. Incubate in PBS for 15 minutes to collect blank injection curves
11. Alto executes automated serial dilutions on cartridge with each lane having a unique concentration using the 900nM stock concentration as designated in *Table 2*

Table 2. List of IgG concentrations used in each channel on Alto

Channel	IgG concentration (nM)
1	0.407
2	1.22
3	3.66
4	11
5	33
6	100
7	300
8	900

12. Introduce IgG for a 3 minute association time followed by PBS-T for a 15 minute dissociation time. Note each channel will be exposed to a single unique concentration, collecting 8 concentrations simultaneously. Serial dilutions are automatically performed on the cartridge from the stock 900nM concentration solution.
13. Regenerate the sensor surface with a 40 second exposure of Glycine-HCl
14. Completion of test. Binding curves fitted to a 1:1 binding model to determine kinetic and affinity constants


(Fig. a)


(Fig. b)

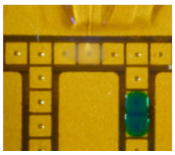

(Fig. c)

Figure 3 a). Microscope images of SPR sensor in Alto Cartridge during experiment. a) Buffer present on SPR sensor to establish baseline. b) IgG sample present on SPR sensor to measure association phase. c) Buffer present on SPR sensor to measure dissociation phase. Each droplet is 700nL in volume, and is rapidly moved back and forth across the sensor surface throughout the measurement.

Results & Discussion

Kinetics and Affinity

The reference corrected curves of IgG binding to Protein A measured with Alto for each of the 8 concentrations is shown in Fig. 4 below. The binding curves demonstrate clear concentration dependence with association and dissociation phases evident. Fitting the data with a 1:1 kinetic model gives an association rate of 2.74e4 1/(M*s), dissociation rate of 8.15e-5 1/s, and an affinity constant of 2.96nM. These results are comparable to other studies of this interaction, and the residuals and errors are all low, indicating high quality binding curves that are similar to ideal theoretical curves.

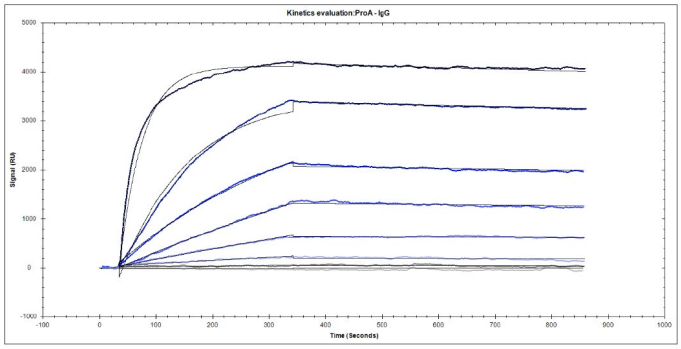


Figure 4. Binding curves measured using Alto for Protein A - IgG for concentrations listed in Table 2.

Table 3. Summary of kinetics and affinity constants determined for Protein A - IgG interaction using Alto

	ka (1/M*s)	k_d (1/s)	KD (nM)
Alto	2.75e4	8.15e-5	2.96

Experiment Sample Volume & Reagent Consumption

The experiment on Alto required a total of 1.125 µL of 900nM IgG analyte, which is only 152ng total. Compared to other SPR instruments, this is 270X less total sample. This substantially reduces the amount of sample required and empowers researchers with limited amounts of sample to obtain high quality kinetic data.

A summary of reagents used in this experiment is shown in *Table 4*.

Table 4. Total quantity of reagents used

Reagent	Alto Volume/Amount Used
IgG	0.152µg
Protein A	0.240µg
10mM Gly-HCl	18µL
Blocking solution	6µL
0.1M EDC	3µL
0.1M NHS	3µL
PBS-T	59.25µL

Experiment Duration

The run time of the entire experiment on Alto was approximately 2 hours. Comparing this to other instruments, this is ~50% faster, demonstrating the improvements in experimental throughput achieved with Alto, not to mention the time saved on sample preparation. Note that the Alto protocol speed was not fully optimized, which when implemented would reduce run time further to ~1 hour.

Conclusion

Alto successfully provided kinetics and affinity of Protein A and IgG, determining an affinity constant of 2.98nM. Alto required 270X less sample and 50% less time than other SPR instruments to perform this analysis. Its unique digital microfluidic platform substantially reduces sample volume and hands on time. The flexible 16 channel design enables high-throughput measurements to be conducted, significantly reducing the time and cost of SPR analysis.