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Kinetic Analysis of High Affinity Antibody Antigen Interaction using Surface Plasmon Resonance

Overview

The determination of kinetic binding constants for antibody-antigen interactions is critical in many research and development applications. OpenSPR allows users to easily and accurately measure the binding kinetics of numerous ligand-antigen systems. In this application note, OpenSPR is used to determine the on and off rates and affinity constant for the interaction between prostate specific antigen (PSA) and its antibody (anti-PSA). Results are compared against industry standard surface plasmon resonance instruments.

Materials & Equipment

- OpenSPR Instrument (SPR-01)
- TracerDrawer Kinetic Analysis Software (TDS)
- Sensor Chip (SEN-AU-100)
- Deionized Water (DI water)
- 1x PBS Buffer pH 7.4, 0.05% Tween 20 (PBS-T)
- 10 mM Sodium Acetate Buffer pH 5.0 (IMB-5.0)
- Regeneration Solution - 5 mM HCl in DI water
- Cysteamine (1 mM in DI water)
- Glutaraldehyde (2.5% in PBS-T)
- Anti-PSA antibody (0.1 mg/mL in IMB-5.0)
- Prostate Specific Antigen (PBS-T with 0.2 mg/ml BSA)
- 1 M ethanolamine solution pH 8 in PBS-T

Safety Notes

Follow the safety precautions outlined in the MSDS for all materials.

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Procedure

The details of the sensor surface preparation can be found in the tech note "Ligand Immobilization using Cysteamine-Glutaraldehyde Functionalization". Once the surface has been prepared with immobilized anti-PSA, the PSA stock solution is diluted into in PBS-T with 0.2 mg/ml BSA. The surface is conditioned by injecting 300 nM PSA four times and regenerating the surface four times with regeneration buffer (5 mM HCl) at 100 µl/min using a 100 µl sample loop. PSA samples at numerous concentrations are prepared and injected at 100 µl/min: 37.5 nM, 75 nM, 300 nM, 600 nM, 1200 nM, and 2400 nM. Regeneration buffer is injected after each PSA sample has cleared the system to clear the bound PSA analyte from the sensor. The generated binding curves are used to extract the kinetic on rate of the PSA binding to anti-PSA. Due to the slow off rate of this interaction, separate extended dissociation off rate experiments are performed. Three separate injections of 300 nM PSA are performed and allowed to dissociate for approximately 60 minutes. An initial control injection of a non-specific protein sample was also performed with 600 nM streptavidin protein. No shift in signal was observed.

Data is post processed in TraceDrawer to determine the on rate, off rate, and affinity. Data was normalized and scaled by 1000 to put into units of picometers. Data was fit with a 1:1 binding model with the off rate fixed to the average as determined by the three extended dissociation experiments.

Results

Kinetic fitting of the PSA binding data produced an off rate of $1.8x10^{-4}$ (+/- $2.1x10^{-6}$) s^{-1} and an on rate of $4.03x10^{4}$ (+/-$9.16x10^{2}$) M*s. This gives an affinity constant (K_D) of 4.5 nM. Results are shown in Figure 1 and 2. These results compare well with other results reported in the literature for the PSA anti-PSA interaction [1].

Repeatability of the sensor response was demonstrated. Between 3 trials of 300 nM, with regeneration in between each injection, the average response was 70pm +/-2.3pm, or a CV of 3.2% (Figure 2).

These results demonstrate the ability of the OpenSPR system to accurately determine kinetic binding rates for antigen antibody interactions.

[1] P. S. Katsamba et al., "Kinetic analysis of a high-affinity antibody/antigen interaction performed by multiple Biacore users," *Analytical Biochemistry,* vol. 352 , pp.208-221, 2006.

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Results

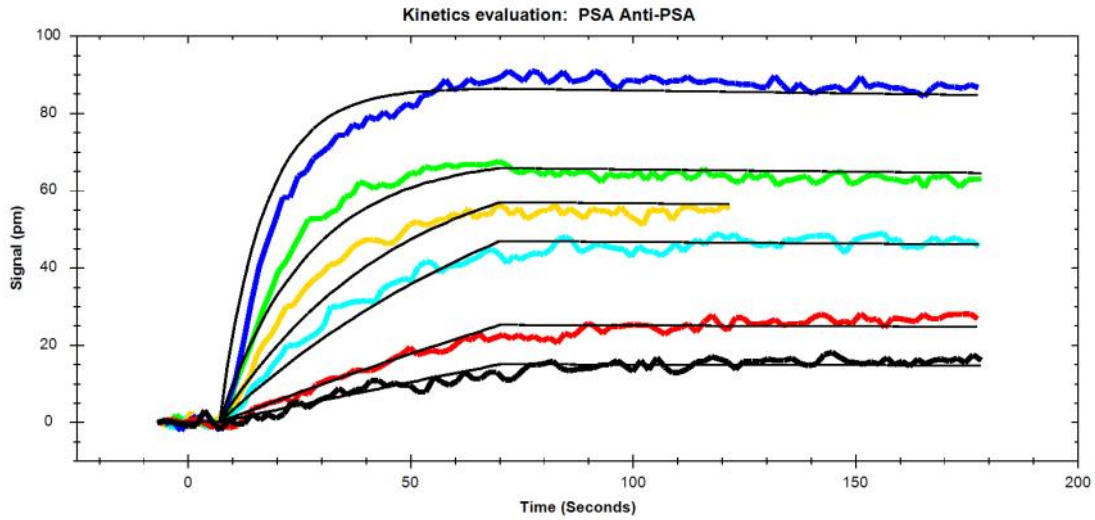

Figure 1. Kinetic analysis of the binding between PSA and anti-PSA on the OpenSPR instrument. Analysis performed in TraceDrawer using a 1:1 binding interaction model. Concentrations (from bottom to top): 37.5 nM, 75 nM, 300 nM, 600 nM, 1200 nM, and 2400 nM

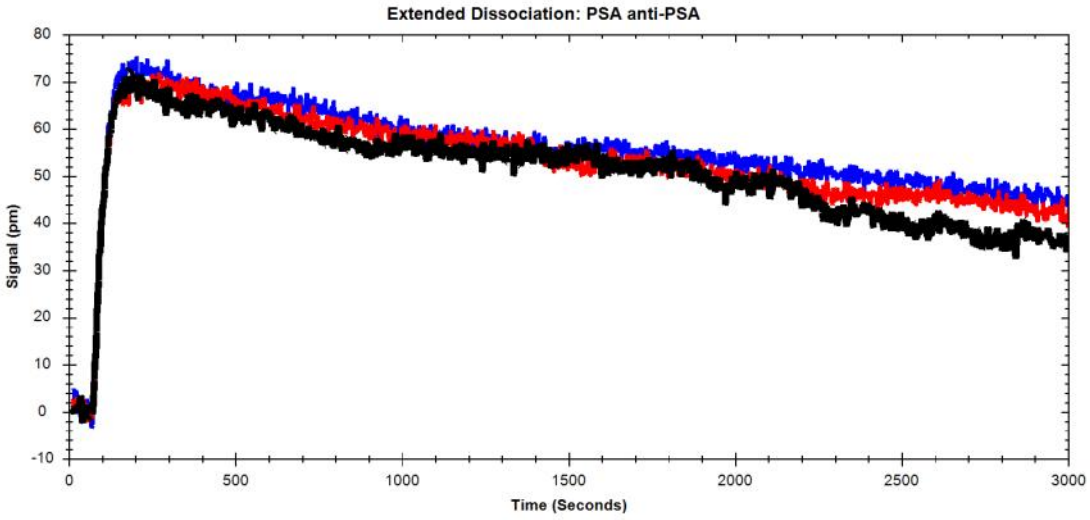

Figure 2. Extended dissociation experiments performed to determine kinetic off rates of PSA anti-PSA system using the OpenSPR instrument. Analysis performed in TraceDrawer. A concentration of 300 nM was used and repeatability was excellent between the three trials. Note this experiment was performed on a separate sensor chip.