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OpenSPR Protein A - IgG Kinetics Experiment Protocol for Biotin-Streptavidin Sensors

Updated: February 2019

1.0 Materials

Quantity	Material	Storage Condition
1	Biotin Sensor	4 °C
1 x 10 µL aliquot	Streptavidin	-20 °C
~ 70 mL	HBS-P Running Buffer	4 °C
1x 6 µL aliquot	66.7 µg/mL Protein A	-20 °C *
1x 10 µL aliquot	0.45 mg/mL Human IgG	-20 °C *
~ 3 mL	10 mM Glycine-HCl pH 1.5	4 °C
	80% Isopropanol	RT
	DI water	RT

* Avoid freeze-thaw cycles. Once thawed, store at 4 °C.

† New syringes should be used for all sample solutions. Syringes from general solutions (i.e. 80% Isopropanol) can be saved and reused.

2.0 Sample Preparation

Preparation of Streptavidin aliquot

1) Dilute one (1) 10 µL aliquot of streptavidin into 200 µL of Running Buffer (HBS-P). Mix well. This will create a solution of **0.5 µM streptavidin**.

Preparation of Human IgG analyte

1. Dilute one (1) 10 µL aliquot of Human IgG into 300 µL of Running Buffer (HBS-P). Mix well. This will make a stock solution of **100 nM IgG**.
2. Prepare serial dilutions of Human IgG using the volumes specified in Table 1. Mix well between each serial dilution.

Table 1 - Serial dilutions of Human IgG

33 nM IgG Solution	11 nM IgG Solution
Combine 100 µL of 100 nM IgG Solution with 200 µL Running Buffer. This will make 300 µL of 33 nM.	Combine 100 µL of 33 nM IgG Solution with 200 µL Running Buffer. This will make 300 µL of 11 nM.

3. Store all prepared samples in a refrigerator or on ice if possible until they are needed for the experiment.

Preparation of Protein A ligand

1. Dilute one (1) 6 µL aliquot of Protein A into 200 µL of Running Buffer (HBS-P). Mix well. This will create a solution of **2 µg/mL Protein A**.

3.0 Test Setup

1. Fill the Buffer 1 bottle with approximately 50 mL of HBS-P Running buffer. Set aside the remaining ~20 mL of HBS-P in a separate container to be used for rinsing the injection port.
2. Fill the Water wash bottle (Bottle 3) with distilled or deionized water.
3. Perform instrument priming using Buffer Bottle 1 with HBS-P. This will take approximately **20 minutes to complete**. During this time, rinse out the sample loop with at least 2 mL of running buffer. You can also prepare the sample dilutions at this time.
4. Once the priming is complete, proceed to load a new Biotin sensor into the OpenSPR following the software prompts.
5. After the sensor is loaded, proceed to follow the bubble removal and prevention procedure using 80% isopropanol. Repeat this procedure until all bubbles are removed from the flow cell.
6. Proceed to start the test.

4.0 Test Procedure

There are 2 options for performing the ligand immobilization depending on how much time you have for your demo.

4.1 Ligand Immobilization – Fast option (10 minutes)

1. Keep the flow rate at 200 uL/min and ensure **CH1 + 2** is selected. Have the response graph showing the **Raw Data**.
2. Perform an injection of **Glycine-HCl** (200 µL or more) to clean the sensor surface.
3. Once the injection is complete and a baseline is established, set the **flow rate to 40 µL/min**.
4. Perform an injection of the **0.5 µM Streptavidin** (200 µL). You should expect to see 1000 – 1500 RU of binding on both channels.

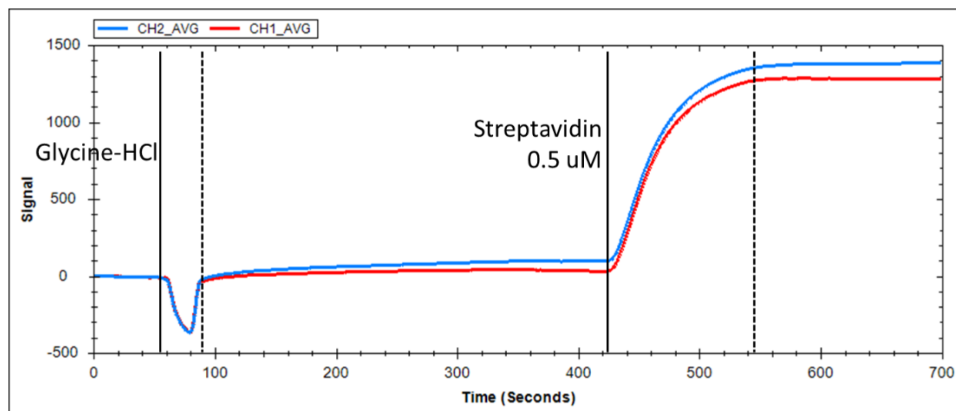

Figure 4.1 Example trace of Streptavidin binding to the Biotin Sensor Chip.

5. Keep the pump speed at **40 µL/min**.
6. Once the new baseline is established after the Streptavidin injection, change the valve position to **CH 2**.
7. **Zero the points** on the graph. This will bring both signals back to a new zero baseline.
8. Perform an injection **2 µg/mL biotin-Protein A** (200 µL).
9. The ligand should immobilize only onto Channel 2 to a net shift of 100-200 RU above the original baseline.

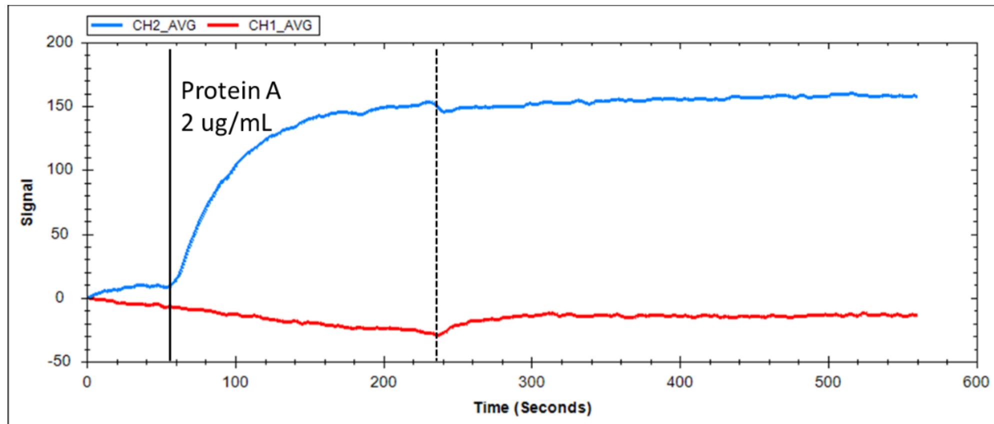

Figure 4.2 Immobilization of biotin-Protein A onto streptavidin-coated sensor.

10. Once the Channel 2 baseline has levelled off, change the valve back to **CH 1+2**.
11. **Zero the points** on the response graph.

4.2 Ligand Immobilization – Full option using Ligand Wizard (15-20 minutes)

1. Have the response graph showing the **Raw Data**.
2. Enter the **Ligand Immobilization Wizard** and select the **Biotin-Streptavidin** sensor chemistry.
3. In the sample details screen, input the following information:

   Molecular weight of Ligand: **34 kDa**

   Molecular weight of Analyte: **150 kDa**

   A minimum immobilization target of 11 RU will be shown.

4. In the Surface Clearing step, perform an injection of **Glycine-HCl** (200 µL or more).
5. In the Surface Preparation step, perform an injection of the **0.5 µM Streptavidin** (200 µL). You should expect to see 1000 – 1500 RU of binding on both channels.

6. For the Ligand Immobilization step, enter the following parameters:

   Ligand name: **Protein A**

Concentration: **2 ug/mL**

Contact Time: **300 s**

Perform an injection of the **2 µg/mL biotin-Protein A** (200 µL).

7. When the injection is complete, click "Next" to move onto the evaluation step.
8. The immobilization level should read 100 – 200 RU (depending on the immobilization amount for the test. The target reached status should say "yes". The ligand should immobilize only onto Channel 2 only.

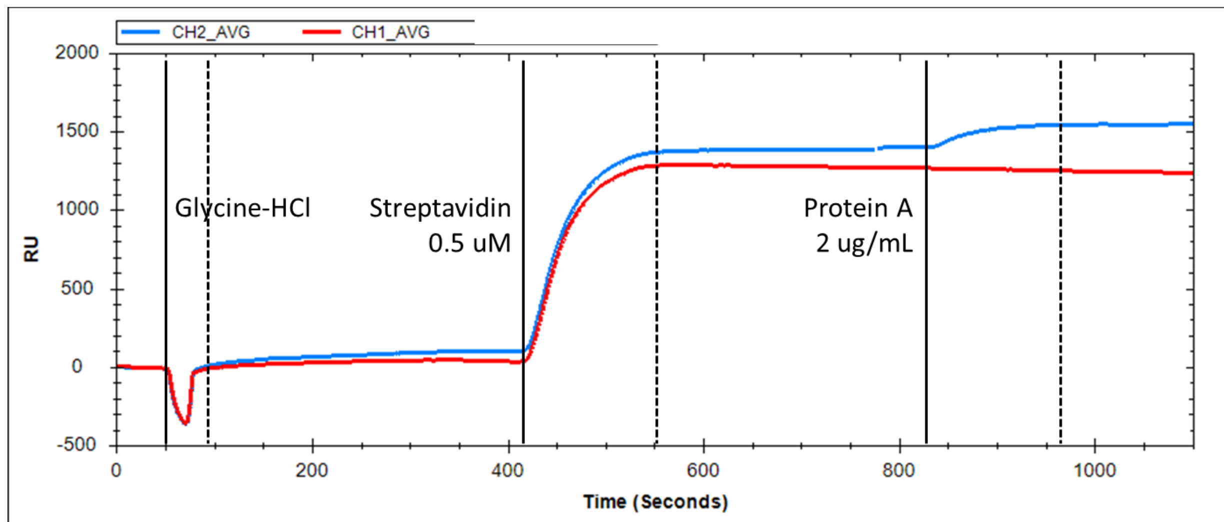

Figure 4.3 Immobilization of Protein A using Biotin-Streptavidin ligand immobilization wizard.

9. Click "Next" to complete the ligand wizard. The graph will automatically zero at this time, and the valves will automatically change the valve back to **CH 1+2**.

4.3 Analyte Analysis

Depending on the amount of time you have for the experiment, you can perform 2 or 3 analyte injections.

1. Keep the valve position in **CH 1+2** for the remainder of the test.
2. Using the glass syringe, perform an injection of **11 nM Human IgG** (150-200 µL). Include the concentration of Human IgG (11 nM) in the sample details. You should expect to see a binding signal ~100 RU.
3. Once the injection is complete, wait at least 3-4 minutes to collect dissociation data (if possible, wait longer).
4. Change the flow rate to **150 µL/min**.
5. Perform an injection of **Glycine-HCl pH 1.5** (200 µL or more). This is a regeneration solution to remove the bound Human IgG from the Protein A. Wait for the signal to baseline after the injection is complete.

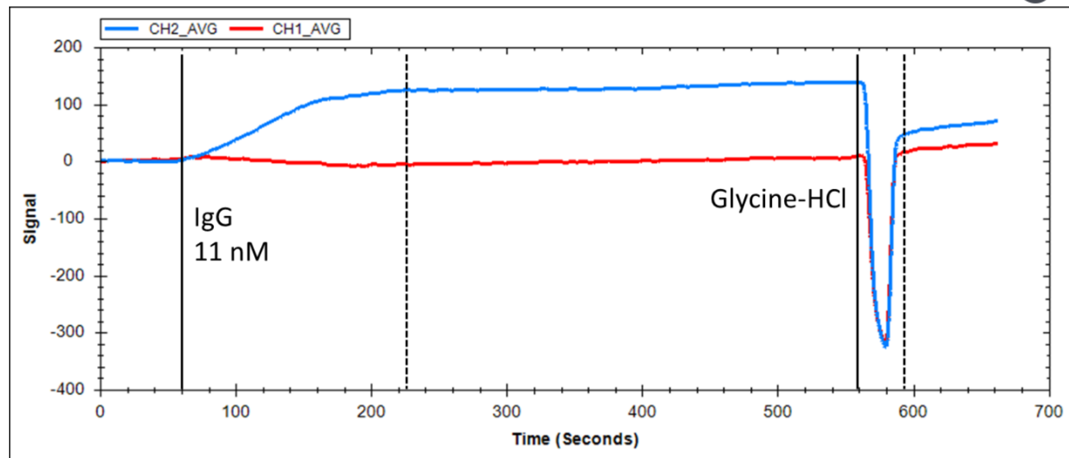

Figure 4.4 First IgG concentration and regeneration with Glycine-HCl.

6. Change the graph view to **Corrected Preview** and keep it on this view for the remainder of the test.
7. Change the flow rate back to **40 µL/min**.
8. Perform an injection of **33 nM Human IgG** (150-200 µL). Include the concentration of Human IgG (33 nM) in the sample details. You should expect to see a higher binding signal compared to the 11 nM.
9. Once the injection is complete, wait at least 3-4 minutes to collect dissociation data (if possible, wait longer).
10. Change the flow rate to **150 µL/min**.
11. Perform an injection of **Glycine-HCl pH 1.5** (200 µL or more) as a regeneration.
12. Change the flow rate to **40 µL/min**.
13. Perform an injection of **100 nM Human IgG** (150-200 µL). Include the concentration of Human IgG (100 nM) in the sample details. You should expect to see a higher binding signal compared the previous concentrations.
14. Once the injection is complete, wait at least 3-4 minutes to collect dissociation data (if possible, wait longer).

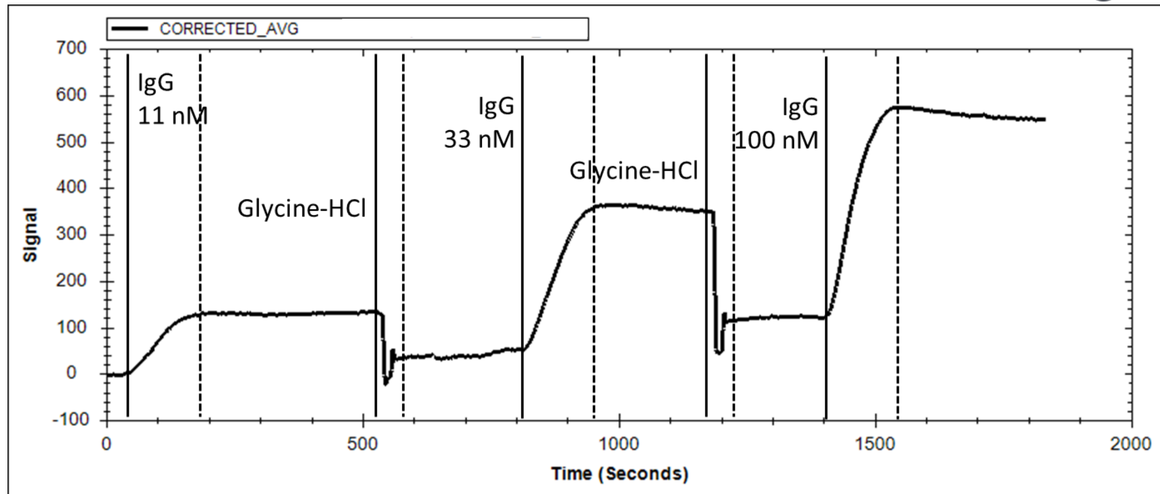

Figure 4.5 Corrected Preview view of 3 concentrations of IgG.

15. Click **Finish** to end the test.

5.0 Kinetic Analysis

1) Fit the data in TraceDrawer using a 1:1 binding model [Figure 5.1]. The measured KD should come out around 1 x 10^{-9} M (approximately from 0.5 - 5 nM).

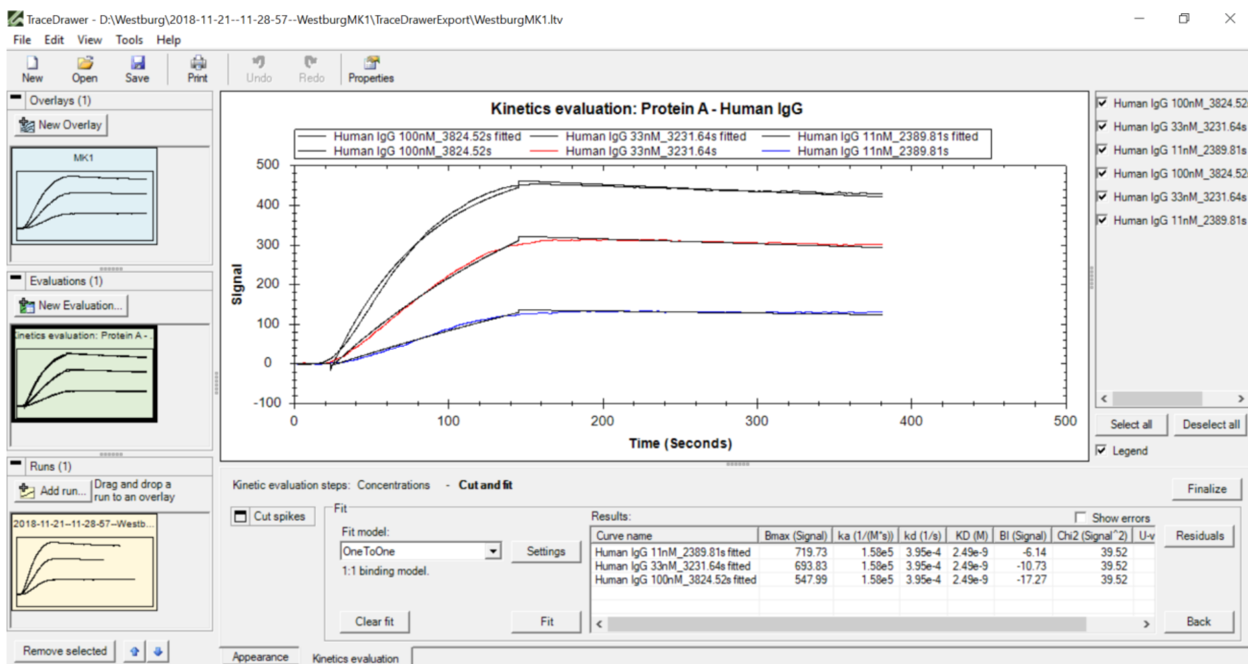

Figure 5.1 Fitted Human IgG curves with resulting KD in the range of 1.0^{-9}.