Kinetics evaluation of Protein A – Human IgG using iMSPR-mini/F & COOH-Au chip (2D)

iMSPR-mini/F is an entry-level model of icluebio providing analysis of protein-protein binding easily and quickly. In this application experiment, a 2D sensor chip, COOH-Au-chip, was used to analyze the binding of Protein A (Ligand protein) and Human IgG (therapeutic antibody), which can be an antibody-drug analysis model. The devices, materials, and conditions used in this experiment can be utilized for various protein-protein binding assays.

Materials

- Instrument: iMSPR-mini/F
- Sensor chip: COOH-Au chip
- Immobilization Reagent: Amine coupling kit (ACK50)
- Immobilization buffer: Acetate buffer pH4.0 (AB40)
- Running buffer: 1xHBST (HB50)
- Regeneration buffer: Glycine-HCl pH1.5 (G15)
- Ligand: Protein A
- Analyte: Human IgG

Procedure

Ligand Immobilization

① Baseline: With inlet tube, 1xHBST is flowed into both channels (ligand channel, reference channel) at a flow rate of 30 ul/min for more than 5 minutes to establish a stable baseline.

② Inject a 1:1 mixture of NHS/EDC into both channels by using the inlet tube at a flow rate of 30 ul/min for 5 minutes followed by 5 minutes of washing in 1xHBST

③ Inject 10 ug/ml 120ul of Protein A (in Acetate buffer pH4.0) only into the ligand channel by using the inlet tube at a flow rate of 30 ul/min for 3 minutes followed by 5 minutes of washing in 1xHBST.

④ Inject 200ul of Quenching buffer into both channels by using the inlet tube at a flow rate of 30 ul/min for 5 minutes followed by 5 minutes of washing in 1xHBST.

⑤ Inject glycine pH 1.5 into both channels by using the inlet tube at a flow rate of 30 ul/min for 2 minutes followed by 30 minutes or more of washing in 1xHBST to stabilize.

Analyte binding

① hIgG was diluted in 1XHBST (on the same day) to a concentration of 100, 50, 25, 12.5 nM, respectively.

② Introduce 200 ul of 100 nM IgG for a 3 minutes association time followed by 1xHBST for a 5 minutes dissociation time.

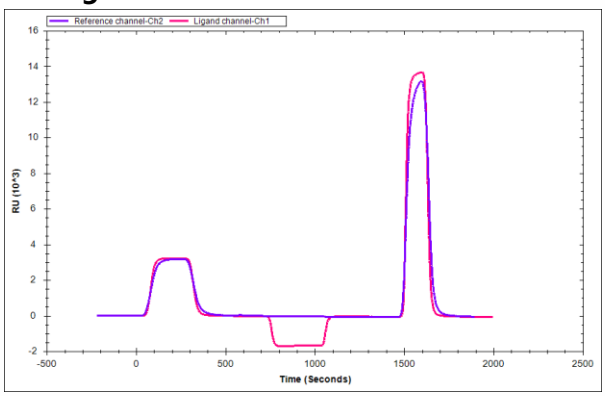
③ After binding, the surface was regenerated by injecting Glycine pH 1.5 solution on the injection valve at a flow rate of 50 ul/min for 2 minutes. And then washed with

1xHBST for 8 minutes to stabilize.

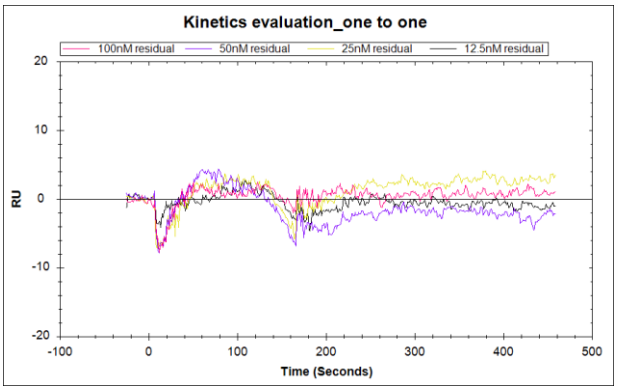
④ Steps ②-③ were repeated for each concentration.

Results

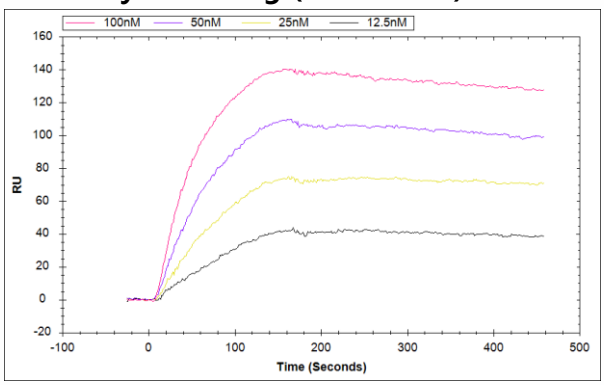
R1. Ligand Immobilization



R4. Residuals



R2. Analyte Binding (Subtracted)



Results summary

Contents	Value
Immobilization Level	50 RU
B_{max}	149.5 RU
K_a (Association rate, 1/M*s)	1.76 x 10^5
K_d (Dissociation rate, 1/s)	3.10 x 10^{-4}
K_D (Affinity)	1.76nM
Chi^2	4.41

R3. Curve fitting: one to one binding model