# Inhibition of Protein-Protein interaction by a small compound using iMSPR-ProX & COOH-Au chip (2D)

Small compound can inhibit binding of protein 1 to protein 2 by competitively binding to the protein 2 binding site of protein 1. In this application experiment, analysis of inhibition of protein 1 – protein 2 binding was performed at various concentrations of a small compound using iMSPR-ProX.

## Materials

- Instrument: iMSPR-ProX
- Sensor chip: COOH-Au chip
- Immobilization Reagent: Amine coupling kit (ACK50)
- Immobilization buffer: Acetate buffer pH5.0 (AB50)
- Regeneration buffer: 10 mM HCl
- Ligand: Protein 2 (6 mg/ml)
- Analyte: Protein 1
- Inhibitor: Small compound

## Procedure

### Ligand Immobilization

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

② Inject a 1:1 mixture of NHS/EDC into both channels at a flow rate of 30 µl/min for 5 minutes followed by 5 minutes of washing in 1xHBST.

③ Inject 60 µg/ml 300 µl of protein 2 (in Acetate buffer pH5.0) only into the ligand channel at a flow rate of 10 µl/min for 20 minutes followed by 5 minutes of washing in 1xHBST.

④ Inject 200 µl of Quenching buffer into both channels at a flow rate of 30 µl/min for 2 minutes followed by 5 minutes of washing in 1xHBST.

⑤ Inject 200 µl of Glycine pH 1.5 into both channels at a flow rate of 30 µl/min for 2 minutes followed by 30 minutes or more of washing in 1xHBST to stabilize.

### Analyte binding

① Prepare 200 µl protein 1 analyte dilutions into the 1XHBST (running buffer) to a concentration of 3.91nM, 7.81nM, 15.6nM, 31.3nM, 62.5nM, 125nM, 250nM, 0 nM.

② Introduce 3.91 nM protein 1 at 30 µl/min for a 3 minutes association time followed by 1xHBST for a 5 minutes dissociation time.

③ After binding, the surface was regenerated by injecting 10mM HCl solution at a flow rate of 30 µl/min for 2 minutes. And then washed with 1xHBST for 6 minutes to stabilize.

④ Repeat ②-③ for each concentration

### Inhibition

① 75 µl of the small compound was diluted in 1XHBST (150 nM, 1% DMSO) in a series of concentrations (200, 100, 50, 0 µM) and incubated for more than 30 minutes. At this time, the DSMO concentrations were maintained at 1%.

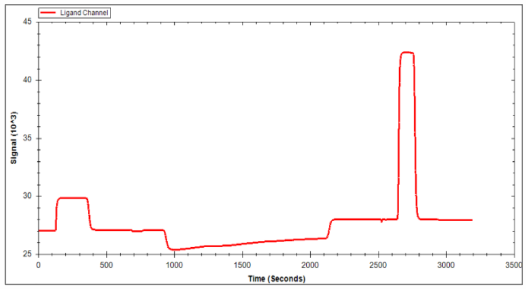
② Introduce mixture of protein 1 (75nM) + small compound 100 µM at 30 µl/min for a 3-minute association time followed by 1xHBST for a 5-minute dissociation time.

③ After binding, the surface was regenerated by injecting 10mM HCl solution at a flow rate of 10 µl/min for 3 minutes. And then washed with 1xHBST for 6 minutes to stabilize.

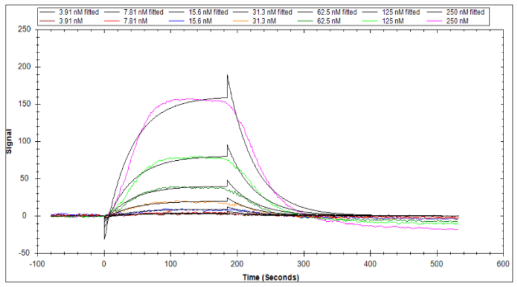
④ Repeat ②-③ for each concentration.

## Results

### R1. Ligand Immobilization



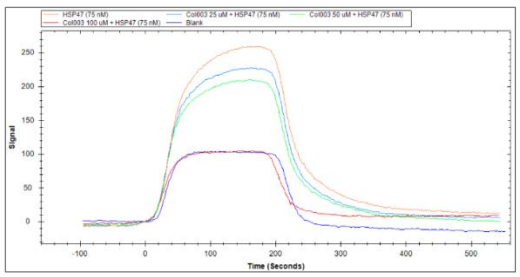

### R2. Protein 1-2 binding Curve fitting: one to one binding model




### T1. Small compound Kinetic Evaluation

| Contents | Value |
|---|---|
| Immobilization Level | 1456.9 RU |
| $B_{max}$ | 14841 RU |
| $K_a$ (Association rate, 1/M*s) | 1.30 x $10^3$ |
| $K_d$ (Dissociation rate, 1/s) | 2.49 x $10^{-2}$ |
| $K_D$ (Affinity) | 1.91 x$10^{-5}$ M |
| $Chi^2$ | 29.97 |

### R3. Inhibition




### Results summary

The appropriate concentration of protein 1 for inhibition analysis was determined through confirmation of protein 1 and 2 binding and kinetic evaluation.

It was indicated that the higher the concentration of the small compound, the lower the signal of protein 1 binding to Collagen. In particular, it can be observed that most of protein 1 and 2 binding is inhibited at 100 μM of the small compound.