

## Protein-peptide Interaction by Surface Plasmon Resonance

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**[Abstract]** This protocol measures the protein-peptide interaction by surface plasmon resonance (SPR) using Biacore X100 (GE Healthcare). The Biacore system can monitor the direct interaction between biomolecules. There are several methods of immobilizing a ligand to the sensor chip. The optimal immobilization method for each experiment needs to be selected. In this protocol, we employed amine coupling to immobilize the protein to the carboxyl-type sensor chip. The procedure generally follows the “Instrument Handbook” of Biacore X100.

### **Materials and Reagents**

1. Purified protein as ligand
2. Synthetic peptide dissolved in DMSO as analyte
3. NaCl
4. NaOH
5. HEPES
6. Tween-20
7. 50 mM NaOH
8. Amine coupling kit {750 mg EDC (N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride), 115 mg NHS (N-hydroxysuccinimide), 10.5 ml ethanolamine-HCl; GE Healthcare, catalog number: BR-10000-50}
9. 10 mM Acetate buffer pH 4 to 5.5 (GE Healthcare, catalog number: BR-1003-49 to 52)
10. DMSO (high grade, such as Sigma-Aldrich, catalog number: 276855, or Sigma-Aldrich, catalog number: D-1435)
11. MilliQ Water
12. Reagents for immobilization (see Recipes)
13. Reagents for binding assay (see Recipes)
14. Ligand (see Recipes)
15. Analyte (see Recipes)

## Equipment

1. CM5 sensor chip (research grade; GE Healthcare, catalog number: BR-1003-99)
2. Biacore X100 Evaluation Software (GE Healthcare)

## Procedure

1. pH scouting (determination of the optimal pH for the ligand)

The positively charged ligand is electrostatically coupled to the negatively charged surface of the sensor chip, leading to ligand concentration. pH scouting is performed so as to determine the pH range that concentrates the ligand.

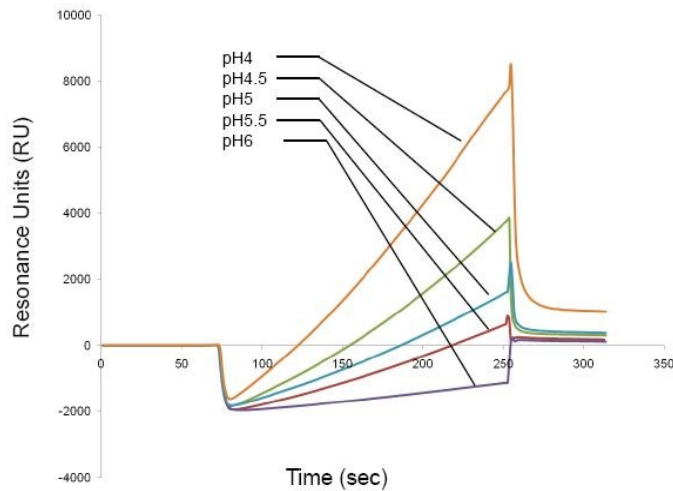
Dilute ligand to a final concentration of 5-200  $\mu\text{g/ml}$  (higher protein concentration is necessary for ligands that are difficult to concentrate) in 10 mM acetate buffer of different pH. A regeneration step using 50 mM NaOH is performed after each pH scouting step.

Below is an example of pH scouting of a ligand to a sensor chip.

Cycle	Reagent	Flow cell	Contact time (sec)	Flow rate ( $\mu\text{l/min}$ )
1	Ligand (12.5 $\mu\text{g/ml}$ in 10 mM acetate buffer, pH 5.5)	2	180	5
2	50 mM NaOH	2	30	10
3	Ligand (12.5 $\mu\text{g/ml}$ in 10 mM acetate buffer, pH 5)	2	180	5
4	50 mM NaOH	2	30	10

2. Immobilization

Prepare the ligand at optimal pH and concentration. The optimal pH is the highest pH that allows ligand concentration in pH scouting. For example, pH5 is the optimal pH for the ligand shown in Figure 1.



**Figure 1. pH scouting**

Below is an example of immobilization of a ligand to a sensor chip.

Cycle	Reagent	Flow cell	Contact time (sec)	Flow rate (µl/min)
1	0.4 M EDC/0.1M NHS*	1**	420	10
2	1 M Ethanolamine	1	420	10
3	0.4 M EDC/0.1M NHS	2	420	10
4	Ligand (12.5 µg/ml in 10 mM acetate buffer, pH 5.5)	2	900	5
5	1 M Ethanolamine	2	420	10

\*

EDC: N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride

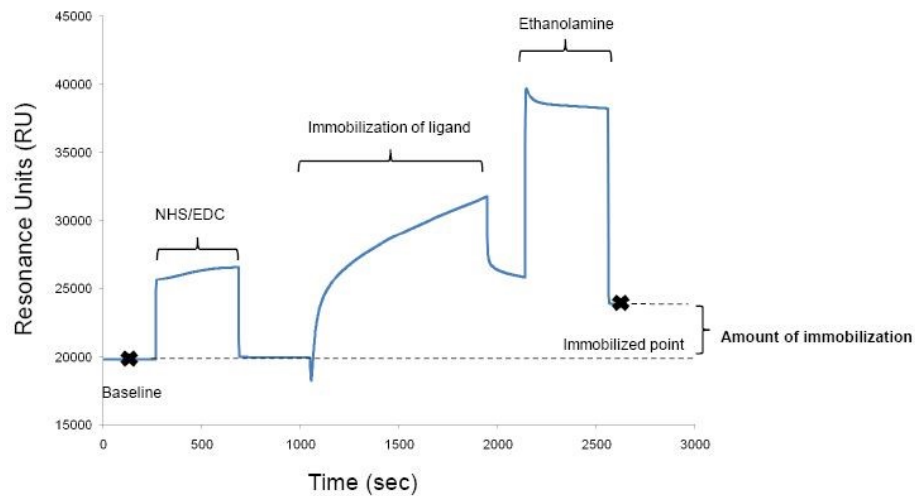
NHS: N-hydroxysuccinimide

These two and ethanolamine are provided in the Amine Coupling Kit.

\*\* Flow cell 1 serves as a reference cell.

After immobilization, run 1x running buffer for immobilization (1x RB-i) at 10 µl/min until the

baseline becomes stable. A typical readout of immobilization is shown in Figure 2.



**Figure 2. Immobilization**

3. Analyte preparation

Precise sample preparation is required for accurate measurement, particularly when the analyte is dissolved in organic solvent. Even a subtle difference of organic solvent concentration between the sample and running buffer leads to inaccuracy.

Below is how we matched the DMSO concentration of our samples.

- a. Dilute 10x HBS buffer with MilliQ water and Tween-20 to 1.1x HBS buffer with 0.11% Tween-20.
- b. Add 25 ml of DMSO and 25 ml of MilliQ water to 450 ml of 1.1x HBS buffer with 0.11% Tween-20 to make 1x running buffer for binding assay (1x RB-b).
- c. Dilute 10 mM peptide in DMSO to 5 mM with MilliQ water, and further dilute to 500  $\mu$ M with 1.1x HBS buffer with 0.11% Tween-20 (this makes 500  $\mu$ M peptide in 1x RB-b).
- d. Dilute 500  $\mu$ M peptide mixture to 12.5  $\mu$ M with 1x RB-b, followed by two-fold dilution to 0.39  $\mu$ M with 1x RB-b.

4. Regeneration condition

For analytes with slow dissociation (when the sensorgram does not go back to the background level at the end of the dissociation step), a regeneration step must be performed after each binding assay. Determine an optimum regeneration condition for each analyte. The regeneration should not change the activity of the immobilized ligand (*i.e.* the responses obtained from the binding assays before and after regeneration should be the same).

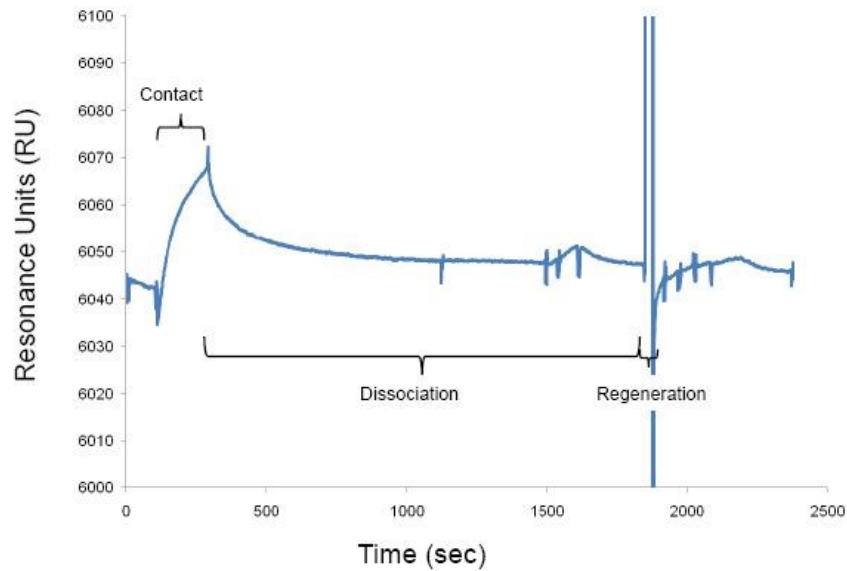
5. Binding assay

Perform the binding assay was with 1x RB-b.

Below is an example of a binding assay.

Cycle	Status	Reagent	Contact time (s)	Flow rate (μl/min)	Flow cell
1	contact	1 x RB-b	180	30	1, 2
2	dissociation	1 x RB-b	800	30	1, 2
3	regeneration	1 mM NaOH	420	30	1, 2
Solvent correction					
1	contact	4% DMSO in 1 x HBS	30	30	1, 2
2	contact	4.5% DMSO in 1 x HBS	30	30	1, 2
3	contact	5% DMSO in 1 x HBS	30	30	1, 2
4	contact	5.5% DMSO in 1 x HBS	30	30	1, 2
5	contact	6% DMSO in 1 x HBS	30	30	1, 2
Cycle	Status	Reagent	Contact time (s)	Flow rate (μl/min)	Flow cell
Analyte loading (repeated for 7 conc. of analyte)					
1	contact	Analyte (peptide) (0 and 0.39 - 12.5 μM)	180	30	1, 2
2	dissociation	1 x RB-b	800	30	1, 2
3	regeneration	1 mM NaOH	420	30	1, 2
Reproducibility check					
1	contact	0.39 μM analyte	180	30	1, 2
2	dissociation	1 x RB-b	800	30	1, 2
3	regeneration	1 mM NaOH	420	30	1, 2
-----					
1	contact	1 x RB-b	180	30	1, 2
2	dissociation	1 x RB-b	800	30	1, 2
3	regeneration	1 mM NaOH	420	30	1, 2

Data analysis is performed with the Biacore X100 Evaluation Software (GE Healthcare). A sensorgram of protein-peptide interaction is shown in Figure 3 and also in the reference (Eguchi *et al.*, 2012).



**Figure 3. Binding assay**

**Recipes**

The buffers used for immobilization and binding must be optimized for every ligand and analyte. The following recipe worked with our protein and peptide samples.

1. Reagents for immobilization
  - 1x running buffer for immobilization (1x RB-i)
  - 10 mM HEPES (pH 7.5)
  - 150 mM NaCl
  - 0.05% Tween-20
2. Reagents for binding assay
  - 10x HBS buffer [100 mM HEPES (pH7.5), 1.5 M NaCl]
  - 1x running buffer for binding assay (1x RB-b)
  - 10 mM HEPES (pH7.5)
  - 150 mM NaCl
  - 0.1% Tween-20
  - 5% DMSO
3. Ligand
  - Purified protein (purity > 90%) equilibrated in 1x RB-i.
4. Analyte
  - Synthetic peptide dissolved in DMSO.

### **Acknowledgments**

This protocol was adapted from Eguchi *et al.* (2012), and generally follows the “Instrument Handbook” of Biacore X100 (GE Healthcare). This work was supported by a Grant-in-Aid for Scientific Research (A, 20248012) from the Japan Society for the Promotion of Science (JSPS), the Research and Development Program for New Bio-Industry Initiatives (2006–2010) of Bio-Oriented Technology Research Advancement Institution (BRAIN), Japan, MEXT-Supported Program for the Strategic Research Foundation at Private Universities, 2011-2015 (S1101035), Sasakawa Scientific Research Grant from The Japan Science Society, and the Institute for Fermentation, Osaka.

### **References**

1. Eguchi, Y., Ishii, E., Yamane, M. and Utsumi, R. (2012). [The connector SafA interacts with the multi-sensing domain of PhoQ in \*Escherichia coli\*](#). *Mol Microbiol* 85(2): 299-313.