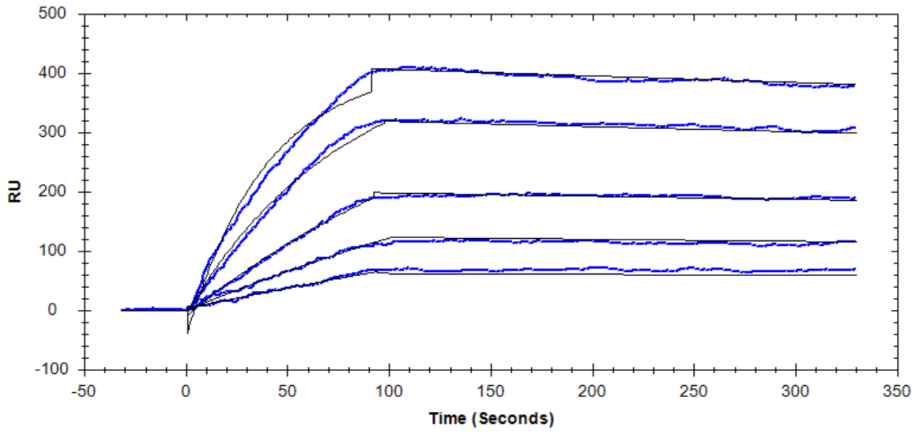
1:1 Binding model for Kinetic evaluation



The sensorgram obtained through the SPR experiment is as above. If kinetic evaluation is performed using these curves, the results in the table below are obtained. This result provides the binding affinity between the two molecules.

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Conc.	Rmax(RU)	k_a(1/Ms)	k_d(1/s)	K_D(M)	RI(RU)	Chi²
8 nM	456.58	1.97E+05	2.83E-04	1.43E-09	3.34	40.70
16 nM					0.50	
32 nM					-8.94	
64 nM					-3.11	
128 nM					-38.87	

The above results were calculated using a 1:1 binding model. In general, the binding affinity between two molecules in the SPR assay is calculated using a 1:1 binding model. 1:1 binding model refers to the binding of one analyte molecule to one ligand molecule. After obtaining the association rate constant (k_a) and the dissociation rate constant (k_d), the equilibrium dissociation constant (K_D) for evaluating affinity can be obtained by dividing the dissociation rate constant by the association rate constant.

The reaction equation between two molecules in a 1:1 reaction is:

$$[L]+[A] \underset{k_d}{\overset{k_a}{\rightleftharpoons}} [LA]$$

The differential equation for the concentration of the Ligand-Analyte complex with time is as follows.

$$\frac{d[LA]}{dt} = k_a[L][A] - k_d[LA] \quad (1)$$

At this time, since the amount of ligand immobilized on the sensor chip is constant, when analyte is injected, the analyte binds to the ligand and the amount of ligand decreases. The ligand concentration can be expressed as

$$[L] = [L]_0 - [LA] \quad (2)$$ $[L]_0$: Initial concentration of ligand

In the association phase, the concentration of LA complex can be expressed as SPR signal (RU), and the initial concentration of ligand can be expressed as Rmax. It is assumed that the concentration of analyte injected is always constant.

If the above conditions are applied, the expression of change in concentration of LA complex with time can be converted as follows.

$$\frac{dR}{dt} = k_a C(R_{max} - R) - k_d R \quad (3)$$ C: Concentration of analyte

By integrating Equation (3), the equation for the SPR signal (Rt) according to time (t) in the association phase can be obtained.

$$R_t = k_a C R_{max}\left[1 - e^{-((Ck_a + k_d)t)}\right] \quad (4)$$

In the dissociation phase, [A]=0 in Equation (1), the following equation can be obtained.

$$\frac{d[LA]}{dt} = -k_d[LA] \quad (5)$$

$$\frac{dR}{dt} = -k_d R \quad (6)$$

The equation for SPR signal (Rt) according to time (t) in the dissociation phase obtained by integrating Equation (6) is as follows.

$$R_t = R_0 e^{-k_a t} \quad (7)$$ R_0: Signal at the end of the association

The curve obtained through the experiment is fitted with Equation (4) in the association phase and Equation (7) in the dissociation phase to obtain the k_a, k_d, and K_D values. In order to obtain reliable values, it is necessary to fit curves at various concentrations.

The values obtained through fitting are as follows.

Association rate constant(k_a): In the biological system, it is usually between 10^2 and 10^7. A higher value means faster association. Because it is a second order reaction, it has units of 1/Ms.

Dissociation rate constant(k_d): In biological systems, it is usually 10^{-1} to 10^{-6}. A lower value means slower dissociation. Since it is a first-order reaction, it has units of 1/s.

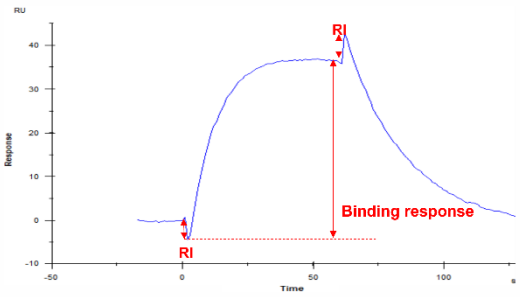
Equilibrium dissociation constant(K_D): The unit of K_D is M(molarity) and K_D is used as a parameter to determine affinity. A lower value means higher binding affinity.

Rmax: This is the maximum signal that can appear when all the ligands have reacted. The theoretical Rmax can be expressed as

$$\text{Rmax} = \frac{\text{Analyte M.W.}}{\text{Ligand M.W.}} \times \text{immobilization level} \times \text{stoichiometric ratio}$$

The above formula is Rmax when all ligands are activated, and in actual experiments, it has a value of 50% or less of the theoretical Rmax.

RI: This is a signal that occurs as a bulk effect. The difference in signal occurs due to the difference in refractive index between the running buffer and the buffer in which the analyte is dissolved. There is a signal difference between the start of the analyte injection and the end of the injection, and the binding signal is determined by excluding this signal.



Chi^2: It is one of the parameters to measure the error between the tested curve and the fitted curve. Calculated as the mean of the squares of the residuals of two curves.

$$\chi^2 = \frac{\sum_{1}^{n}(r_f - r_x)^2}{n - p}$$

r_f is the fitted value at a given point
r_x is the experimental value at the same point
n is the number of data points
p is number of fitted parameters