

Procedure

Two-site steady-state binding model in Biacore T200

The steady-state model for evaluating affinity constants is based on the equation for equilibrium binding in a 1:1 interaction system:

$$\text{Response} = (\text{Conc} \times R_{\text{max}} / [\text{Conc} + K_D]) + \text{RI}$$

Response (RU) = Response level of analyte

Conc (M) = Concentration of analyte

R_{max} (RU) = Maximum binding capacity

K_D (M) = Equilibrium dissociation constant

RI (RU) = Bulk refractive index contribution in the sample

The term RI is an offset to permit fitting to curves that do not pass through the origin. A strict 1:1 model equation has zero response at zero concentration. The model for simultaneous binding to two sites, where the response is the sum of two independent binding terms is:

$$\text{Response} = (\text{Conc} \times R_{\text{max}1} / [\text{Conc} + K_{D1}]) + (\text{Conc} \times R_{\text{max}2} / [\text{Conc} + K_{D2}])$$

The offset term is not included in the two-site model because fitting steady-state data to a multisite model with an offset term tends to be unstable when the number of experimental points is low.

Follow the steps below to create the model or import a predefined model from the Biacore T200/Biacore T100 Evaluation Software.

Create a model

1. Open Biacore™ T200/Biacore T100 Evaluation Software.
2. Choose **Tools:Models:Affinity**. Select the **Steady State Affinity** model and click on **New**. Use the existing model as a template.
3. Enter a name and description for the model.
4. Replace the expression in the **Formula** box with the corresponding expression for the two-site binding equation. Remember to remove the offset term.
5. Edit the list of parameters according to the terms in your new formula.
 - Replace R_{max} with $R_{\text{max}1}$ and $R_{\text{max}2}$, and K_D with K_{D1} and K_{D2} .

- Use $Y_{\text{max}}/2$ as the initial value for both the $R_{\text{max}1}$ and $R_{\text{max}2}$ terms. This represents the assumption that each of the two sites contributes to half the analyte binding capacity of the surface.
- Use $X_{\text{max}}/10$ as the initial value for both the K_D terms. This allows the initial value to adapt automatically to the scale of analyte concentrations.
- Delete the offset parameter.

6. Edit the report content according to the parameters in your new formula.

Name	Type	Initial value	Allow negative value	Description
KD1	Fit global	XMax/10	☐	KD for first site
Rmax1	Fit global	YMax/2	☐	Rmax for first site
KD2	Fit global	XMax/10	☐	KD for second site
Rmax2	Fit global	YMax/2	☐	Rmax for second site

Name	Value
KD1 (M)	KD1
Rmax1 (RU)	Rmax1
KD2 (M)	KD2
Rmax2 (RU)	Rmax2

7. Click **OK** to save the model.

Import a model

1. Open Biacore T200/Biacore T100 Evaluation Software.
2. Choose **Tools:Models:Affinity**. Click on **Import** and browse for the **Two-site affinity** model and click on **Open**.
3. In the **Import Models** window select the **Two-site affinity** model and click on **Add =>**. Click on **Import** to add the model to the current models.

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CY15005-16Oct20-PD

