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:

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

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:

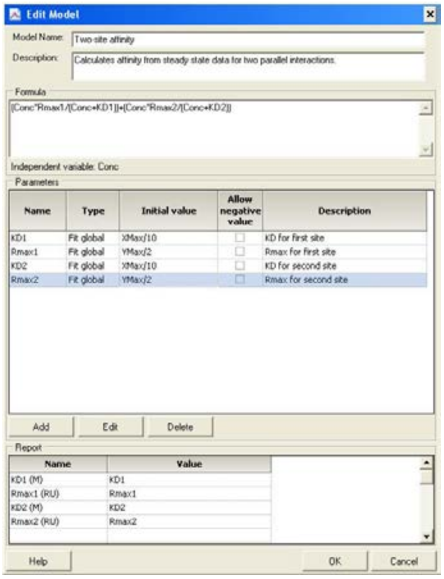
$$\text{Response} = (\text{Conc} \times R_{max1}/[\text{Conc} + K_{D1}]) + (\text{Conc} \times R_{max2}/[\text{Conc} + K_{D2}])$$

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_{max1} and R_{max2}, and K_D with K_{D1} and K_{D2}.
   - Use $Y_{max}/2$ as the initial value for both the R_{max1} and R_{max2} terms. This represents the assumption that each of the two sites contributes to half the analyte binding capacity of the surface.
   - Use $X_{max}/10$ 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.



Model Name: Two-site affinity
Description: Calculates affinity from steady state data for two parallel interactions.

Formula

```
(Conc*Rmax1/(Conc+KD1))+(Conc*Rmax2/(Conc+KD2))
```

Independent variable: Conc

Parameters

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

Report

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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