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:

Response = (Conc × R_{max} /[Conc + K_n]) + 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:

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

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.
- 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 a	lfinity				
Description:	Calculates	Calculates attinity from steady state data for two parallel interactions.				
Formula]]+[Conc"Rmax2/]Conc-	10.00			
leone nuide	infeation and	Reference summer former	HUNS II			
	variable: Con	c				
Parameters	18	1	Allow			
Name	Туре	Initial value	negative value	Description		
KD1	Fit global	XMax/10	0	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		
Add	Ed	1				
Report	-	1				
Report	ne	R Delote Value			-	
Report Nan KD1 (M)	ne	R Delete Value			-	
Report Nan KD1 (M) Rmax1 (RU)	ne k	R Delote Value				
Report	ne k	R Delete Value D1 tmax1				
Report Nan KD1 (M) Rmax1 (RU) KD2 (M)	ne k	R Delete Value D1 D1 D2			2	

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

Import a model

- 1. Open Biacore T200/Biacore T100 Evaluation Software.
- Choose Tools:Models:Affinity. Click on Import and browse for the Two-site affinity model and click on Open.
- 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

