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Biacore S200

Software Handbook

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1 Introduction

About this chapter

This chapter provides an overview of the Biacore™ S200 system and an introduction to the terminology used with Biacore systems.

The chapter contains the following sections:

- System overview
- Biacore terminology
- Associated documentation

System overview

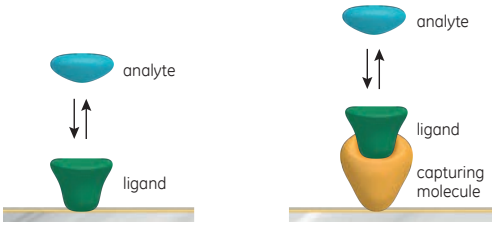
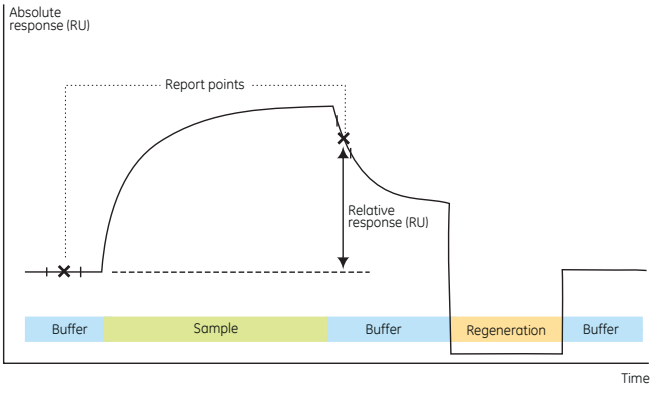
The Biacore S200 system is a high performance system for analysis of biomolecular interactions, based on surface plasmon resonance (SPR) technology. The Control Software supplied with the system offers flexible facilities for creating and editing analysis methods. Results are evaluated in separate Evaluation Software with dedicated functions for common applications.

Instrumentation in the Biacore S200 system is described in the *Biacore S200 Operating Instructions*. Important features relevant to software operation include:

- The Biacore S200 system supports simultaneous analysis in up to four flow cells (numbered Fc1 to Fc4) connected in series. The flow cells are arranged in pairs (Fc1-2 and Fc3-4) with minimum dead volume between the flow cells in a pair to provide accurate reference subtraction.
- The sample compartment accommodates one microplate (96- or 384-well, regular or deep-well) and one reagent rack for reagent vials. A combined sample and reagent rack can be used in place of the separate microplate and reagent rack.
- The temperature in the sample compartment is controlled separately from the analysis temperature, allowing samples to be kept at one temperature while analysis is performed at another. Samples equilibrate to the analysis temperature during injection into the flow cell. The analysis temperature can be varied during a run, and the sample compartment temperature can be set to follow the analysis temperature if desired.
- The system includes a buffer selector valve, allowing analysis to be performed in up to four different buffers in the same unattended run.

Biacore terminology

Biacore monitors the interaction between two molecules, of which one is attached to the sensor surface and the other is free in solution. Essential terms used in the context of work with Biacore system are explained in the table below.

Term	Explanation
Ligand	The interaction partner that is attached to the surface. Attachment may be either covalent or through high affinity binding to another molecule (the ***capturing molecule***) which is in turn covalently attached to the surface.  ***Note:*** *The term "ligand" is applied here in analogy with terminology used in affinity chromatography contexts, and does not imply that the surface-attached molecule is a ligand for a receptor.*
Analyte	The interaction partner in solution.
Regeneration	The process of removing bound analyte from the surface after an analysis cycle in preparation for a new cycle.
Resonance units (RU)	The unit used to measure SPR response. The response is directly proportional to the mass concentration of molecules on the surface.
Sensorgram	A plot of response against time, showing the progress of the interaction. Sensorgrams may be analyzed to provide information on the rates and extent of the interaction. A particular sensorgram may also be referred to as a *curve*. 

Term	Explanation
Active surface	Commonly, sample is passed over two or more flow cells in series, where the first flow cell is prepared without ligand and serves as a reference. Surfaces with ligand are referred to as *active* surfaces.
Reference-subtracted curve	The curve obtained when the sensorgram from a reference surface is subtracted from that from the active surface. Reference-subtracted curves are designated by flow cell number, for example ***Fc2-1***.
Report point	A specified point on a sensorgram, which records the response averaged over a short time window. The response may be absolute (above a fixed zero level determined by the detector) or relative to the response at another specified report point.
Relative response	The response at one report point relative to that at another. Frequently, responses are quoted as relative responses in relation to a baseline set at the beginning of the cycle. ***Note:*** *Do not confuse relative response and reference-subtracted response. Reference subtraction provides the difference between the response in two flow cells and is applied to all points in a sensorgram. Relative response refers to the difference between the response at two points in the same sensorgram.*

Associated documentation

This Handbook describes the Biacore S200 Control Software and Evaluation Software, version 1.0. The *Biacore S200 Operating Instructions* provide you with the instructions needed to operate and maintain Biacore S200 in a safe way. All users must read the entire Operating Instructions before installing, operating or maintaining the product.

General handbooks and documentation describing the technology are available from Cytiva.

2 Control Software: basic operation

About this chapter

This chapter describes the basic operation of Biacore S200 Control Software.

In this chapter

Section		See page
2.1	Starting the Control Software	9
2.2	Software preferences	13
2.3	Performing a run	15
2.4	Template and result files	17

2.1 Starting the Control Software

Starting the software

Start the Control Software from Windows. Operations that can be performed in the software depend on whether the computer is connected to a powered instrument or not.

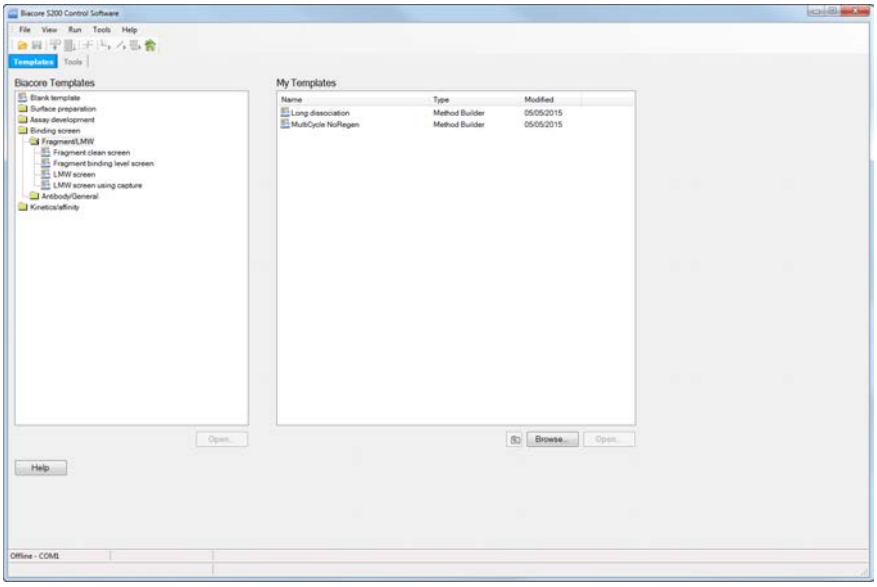
Status	Functions
Connected	Communication will be established and the instrument will be controlled from the Control Software.
Not connected (or instrument not powered)	The software can be used to create and examine method templates and to open result files. Functions that act directly on instrument activity will not be available.

The start screen has two tabs, providing access to templates and tools. The software opens on the ***Templates*** tab by default.

Click the ***Home*** button ( ) on the toolbar or choose ***Run*** →***Template*** from the main menu at any time (except during an ongoing run) to close the current window and return to the start screen.

Note: *Run:Template is only available when the software is connected to an instrument.*

Templates

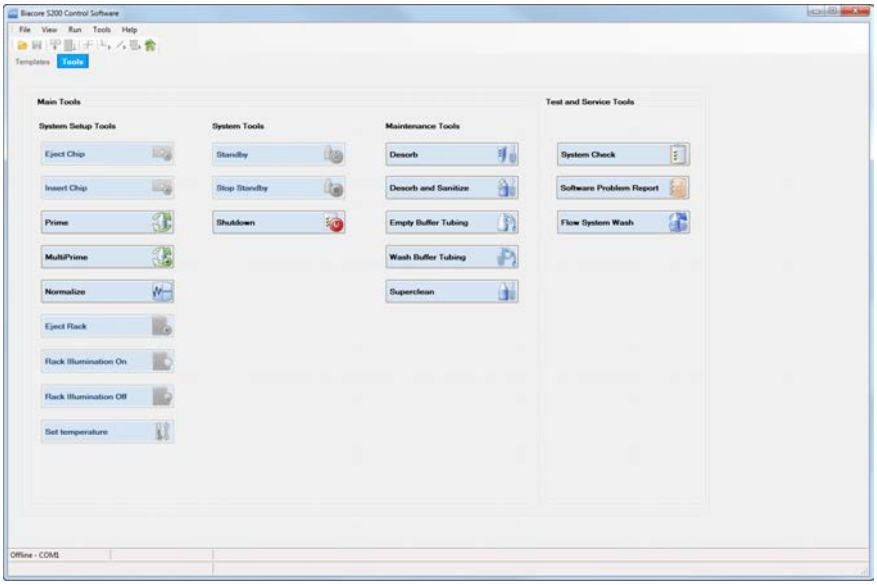


Predefined Biacore templates are listed in the left panel of the the ***Templates*** tab and custom templates in the right panel.

Panel	Description
Biacore templates	Predefined Biacore templates for a range of application purposes are provided with the software. The templates may be run directly after providing sample information, or they can be used as a starting point for developing custom templates. Biacore templates cannot be overwritten. Any modifications must be saved in custom templates.
My templates	This panel lists custom templates stored in the default templates folder (see *Folders preferences, on page 13*). Click ***Browse*** to access templates stored elsewhere.

Double-click a template or select a template and click ***Open*** to open the template.

Tools



Tools are divided into groups according to their usage and function.

The tools can also be accessed from the ***Tools*** option on the main menu.

System Setup Tools

See the *Biacore S200 Operating Instructions* for more details of system setup tools.

Tool	Description
Eject Chip	Undocks the sensor chip.
Insert Chip	Docks the sensor chip.
Prime	Flushes the flow system once with fresh buffer.
MultiPrime	Flushes the flow system a specified number of times with fresh buffer.
Normalize	Adjusts the detector response to compensate for slight differences between individual sensor chips.
Eject Rack	Ejects the rack tray from the sample compartment
Rack Illumination On	Switches the sample compartment illumination on.[1]
Rack Illumination Off	Switches the sample compartment illumination off.[1]
Set Temperature	Sets the sample compartment and analysis temperature.

[1] Use the Tools option in the main menu to turn rack illumination on or off during a run.

System tools

System tools are described in the *Biacore S200 Operating Instructions*.

Tool	Description
Standby	Puts the instrument in standby mode. Standby mode is started automatically at the end of a run. Use this tool to restart standby mode if it has been stopped.
Stop Standby	Stops standby mode.
Shutdown	Shuts down the Biacore S200 instrument. Use this tool if you intend to leave the instrument unused for more than 7 days.

Maintenance Tools

Maintenance tools are described in the *Biacore S200 Operating Instructions*.

Tool	Description
Desorb	Cleans the flow system.

Tool	Description
Desorb and Sanitize	Cleans and disinfects the flow system.
Empty Buffer Tubing	Washes and empties the buffer tubing.
Wash Buffer Tubing	Washes the buffer tubing.
Superclean	Cleans the flow system using harsher cleaning agents than ***Desorb***.

Test and Service Tools

Test and Service tools are described in the *Biacore S200 Operating Instructions*.

Tool	Description
System Check	Performs a comprehensive check of system performance.
Software Problem Report	Compiles a report detailing the computer software environment, for submission to Cytiva in troubleshooting situations.
Flow System Wash	Flushes the flow system with buffer at a high flow rate to clear obstructions such as aggregated particles.

2.2 Software preferences

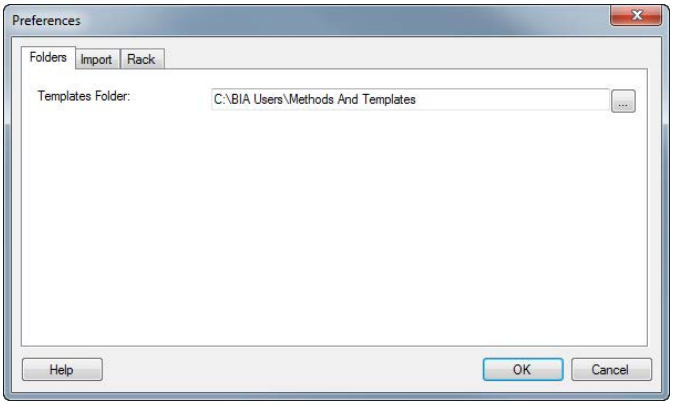
Accessing preferences

Choose ***Tools*** →***Preferences*** from the main menu to set preferences for the Control Software. Preferences are organised on three tabs:

- ***Folders*** preferences
- ***Import*** preferences
- ***Rack*** preferences

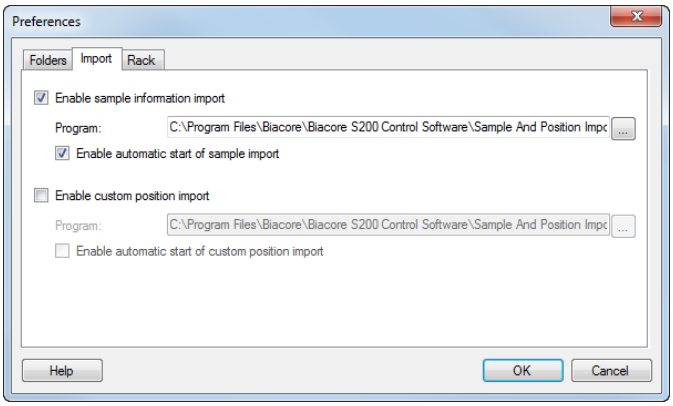
Note: *The **Preferences** settings apply to all users of the software on the current computer.*

Folders preferences



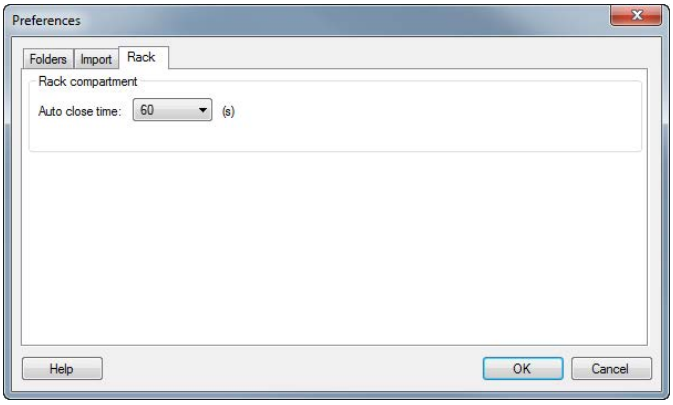
The setting on this tab determines the default folder for method templates. The folder ***C:\BIA Users\Methods and Templates*** is selected by default. Use the browse button (...) to choose an alternative folder.

Import preferences



The settings on this tab determine the facilities for importing data to the sample table. Details of sample import facilities are described in *Appendix A.2 Importing data, on page 197*.

Rack preferences



The setting on this tab determines the time interval before the sample rack is automatically retracted into the instrument after being ejected. See the *Biacore S200 Operating Instructions* for further information.

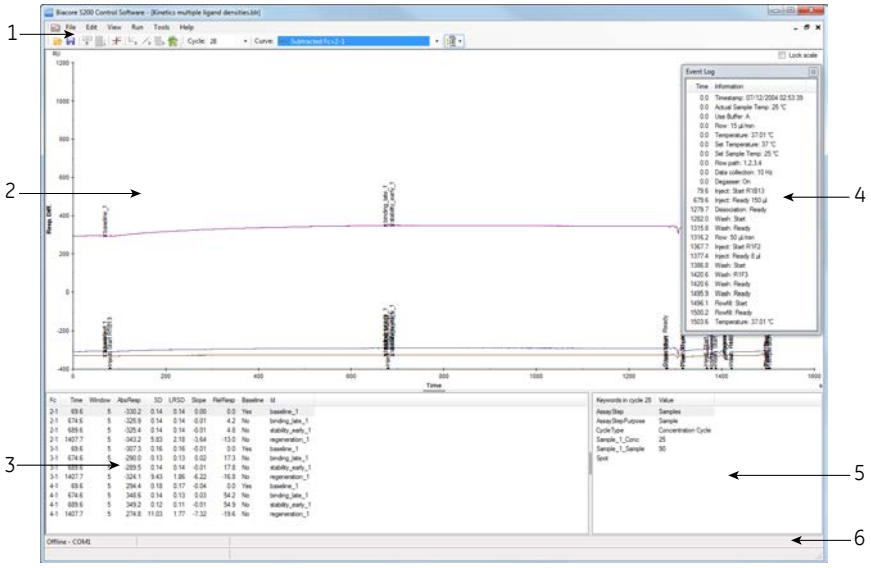
2.3 Performing a run

Starting a run

Runs may be performed under either manual or automated control. Instrument and experiment preparations before starting a run are described in the *Biacore S200 Operating Instructions*.

Mode	Description
Manual	Manual run provides interactive control of instrument operation, executing commands one by one as they are issued. This mode is most useful for ad hoc experiments involving one or a few injections, such as testing the response obtained from injection of a single sample. Choose ***Run*** →***Manual Run*** from the main menu to start a manual run. See *Chapter 3 Manual run, on page 18* for further details.
Automated	Automated runs are controlled from methods (described in *Chapter 5 Methods, on page 42*). Start the run from the method template.

Screen display during a run



Region	Function
1	The menu and toolbar provide access to control commands. Refer to the on-line help for details of menu and toolbar options.
2	The sensorgram window displays the sensorgrams for the current run or the currently open file.[1]
3	The report point table lists report points for the currently displayed cycle. Report points record the response at a set time and are defined automatically: custom report points can also be added in methods, or after the run in either the Control Software or the Evaluation Software.
4	The event log records settings at the start of the run and instrument control events during the run. The event log is displayed in a separate window, opened by clicking on the Event Log button at the right of the toolbar.
5	The keyword table lists keywords for the currently displayed cycle. Keywords are set automatically: custom keywords can be added in the Evaluation Software.
6	The status bar displays the instrument status, including the temperature of the detector and the sample compartment, the elapsed run time and the estimated total run time for automated runs.

[1] During a run, there is a short delay between the appearance of events and updating the sensorgram data. This is normal.

Display options

Use the ***Cycle*** selector on the toolbar to choose which cycle to display. During an ongoing run, the display will revert to the current cycle when a new cycle is started.

Use the ***Curve*** selector on the toolbar to set the current curve.

Use the options in the ***View*** menu to control how data is displayed. Refer to the on-line help for details of the options.

Finishing a run

Issue a ***Stop Run*** command to stop a manual run (see *Chapter 3 Manual run, on page 18*).

Automated runs end automatically when the end of the method is reached.

See the *Biacore S200 Operating Instructions* for clean-up procedures after a run.

2.4 Template and result files

Templates

Immobilization templates are saved in files of ftype ***.bwlmmob***.

Method templates are saved in files of type ***.Method***.

Templates may be saved in any location. The default location is specified in ***Tools →Preferences*** (see *Folders preferences, on page 13*).

Result files

Results are saved in files of type ***.blr***. Result files from automated runs contain a complete copy of the method template as well as the results of the run.

3 Manual run

About this chapter

This chapter describes how to perform a manual run in Biacore S200.

In this chapter

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3.1 Introduction

Description

Manual run allows you to control a run interactively. All settings except temperature and choice of microplate and/or reagent rack can be changed during the run. Commands are placed in a queue if the instrument is busy when a command is issued: queued commands that have not yet been started can be edited or deleted from the queue.

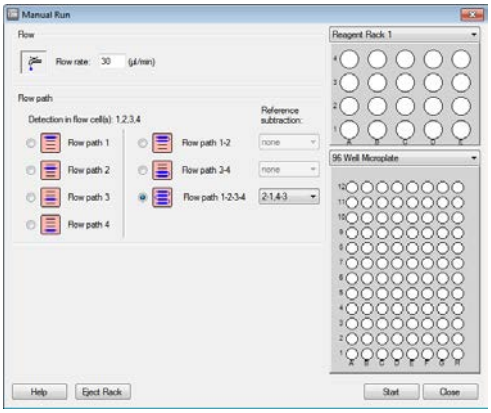
Results of a manual run

The results of a manual run are saved in a normal result file, and can be evaluated in the Evaluation Software. There are however no predefined keywords associated with the run, and the results cannot be evaluated with application-specific Evaluation Software tools.

3.2 Starting a manual run

Procedure

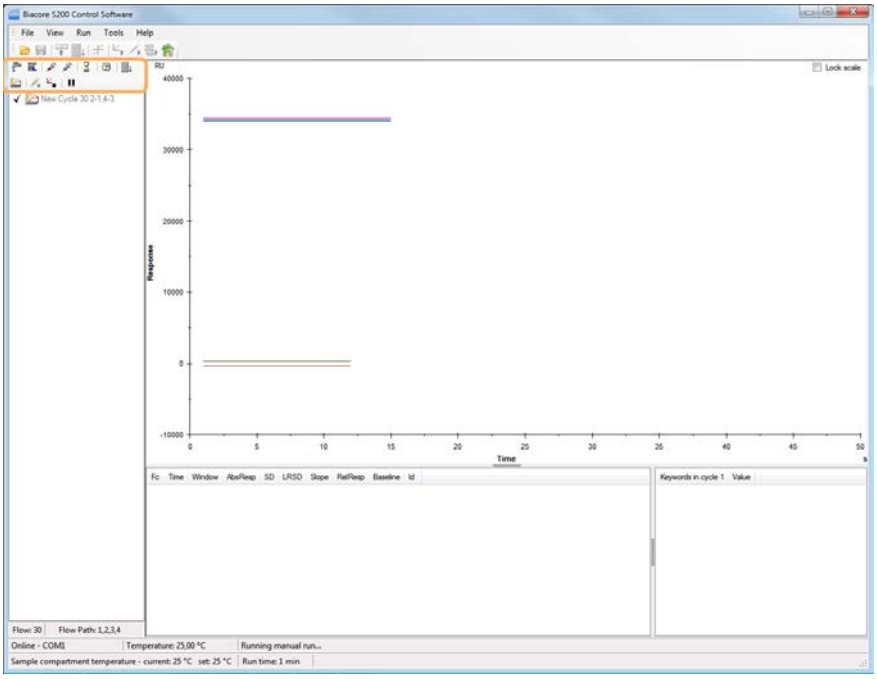
Follow the steps below to start a manual run.

Step	Action
1	Choose ***Run*** →***Manual Run*** from the main menu. Result: The ***Manual Run*** window opens. 
2	Choose the initial settings for flow rate, flow path and reference subtraction. You can change the flow rate at any time during the run. You can change the flow path at any time: during a cycle, the available options are restricted by the choice made when the cycle is started.
3	Choose the rack and microplate settings. These settings will apply throughout the run and cannot be changed.
4	Click ***Eject Rack*** to eject the rack tray so that you can load your samples.
5	Click ***Start*** to start the run. You will be asked to specify a result file name before the run actually starts.

3.3 Controlling a manual run

Presentation

The screen presentation for a manual run is similar to that for an automated run (see *Screen display during a run, on page 15*) with the addition of a panel for the command queue at the left. The run is controlled from the command buttons (highlighted in the illustration below) in the queue panel or the options in the ***Command*** menu:.



Commands are executed immediately if the instrument is idle. With a few exceptions (noted in the detailed descriptions below), commands issued when the instrument is busy are placed at the end of a queue. The queue is listed in the left-hand panel, with commands that have been executed in gray text and those that are pending in black text. The command currently being executed is marked with a "working" icon ( ).

Managing commands

Right-click on a pending command to access a menu with options for:

- editing the command
- inserting a new command before the selected command (you choose the command to insert from a dialog box)
- deleting the command

You can also use the right-click menu to copy selected commands and paste them elsewhere in the queue. The ***Copy*** function works with both completed and pending commands.

Manual run commands

Available commands are described in the table below.

Button	Command	Description
	***Flow rate***	Sets the flow rate to a new value.
	***Flow path***	Changes the flow path. During a cycle, you can only select a flow path within a range allowed by the setting chosen when the cycle was started (for example, if the current setting is ***Flow path 1-2***, you cannot extend it to ***Flow path 1-2-3-4***).
	***Sample injection***	Injects sample or reagent. Choose the position from which the sample will be taken and specify a contact time. Positions that can be chosen are determined by the rack settings in the manual run start-up dialog. Make sure that the chosen position contains enough sample for the injection. The required volume for the specified contact time is indicated in the dialog box.
	***Regeneration injection***	Injects regeneration solution. This command is identical to ***Sample injection*** except for the addition of a ***High viscosity solution*** option. Check this option if your regeneration solution has a relative viscosity higher than about 3 (corresponding to about 35% glycerol or 40% ethylene glycol at 20°C). This will adjust the injection procedure to ensure correct handling of viscous solutions, and will limit the maximum contact time that can be specified.
	***Wait***	Inserts a ***Wait*** command in the queue, causing the instrument operation to pause for the specified time period. Buffer continues to flow over the sensor surface during the ***Wait*** period and data collection continues.

Button	Command	Description
	***Eject rack tray***	Ejects the rack tray so that you can load more samples. Do not change the type of microplate or reagent rack on the tray. This command is inserted immediately after the command currently under execution, so that the rack tray will be ejected as soon as the current command is completed. If you want to place the command later in the queue, use the right-click menu in the queue panel to insert the command at the appropriate place.
	***New Cycle***	Starts a new cycle. You can choose a new flow path and reference subtraction setting for the new cycle, independently of the setting in the current cycle.
	***Stop <command>***	Stops the command currently being executed. The icon changes to show the command that will be stopped, or is gray if the current command cannot be stopped (e.g. it is not possible to stop an ***Eject Rack Tray*** command).
	***Stop Run***	Finishes the run.
	***Pause Run***	Pauses the run until a ***Resume Run*** command is issued. Buffer continues to flow over the sensor surface while the run is paused. Data collection continues during the pause.
	***Resume Run***	Resumes a run that is paused.
	***Add Report Point***	Adds a report point to the sensorgram.
	***Help***	Displays help for the manual run.

3.4 Ending a manual run

Procedure

Follow the steps below to end a manual run.

Step	Action
1	Issue a ***Stop Run*** command. The command will normally be placed at the end of the queue. If you want to stop the run before the queue is completed, use the right-click menu in the queue panel to delete commands from the queue or to insert the ***Stop Run*** command in the appropriate position.
2	Choose ***Eject Rack*** to eject the rack tray and remove your samples and reagents.
3	Choose ***Eject Chip*** to undock the chip if desired.

4 Sensor surface preparation and assay development

About this chapter

This chapter describes how to determine a suitable pH for ligand immobilization, attach ligand or capturing molecule to the sensor surface and scout for appropriate assay conditions.

Note: *The immobilization procedure is used only for permanent attachment of molecules to the surface. Reversible attachment by high affinity capture is performed as part of the analysis method (see* *Capture injection, on page 59**).*

In this chapter

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4.1 Immobilization pH scouting

Introduction

Before performing ligand immobilization, you may need to run a pH scouting experiment to determine a suitable pH for immobilization.

Running pH scouting

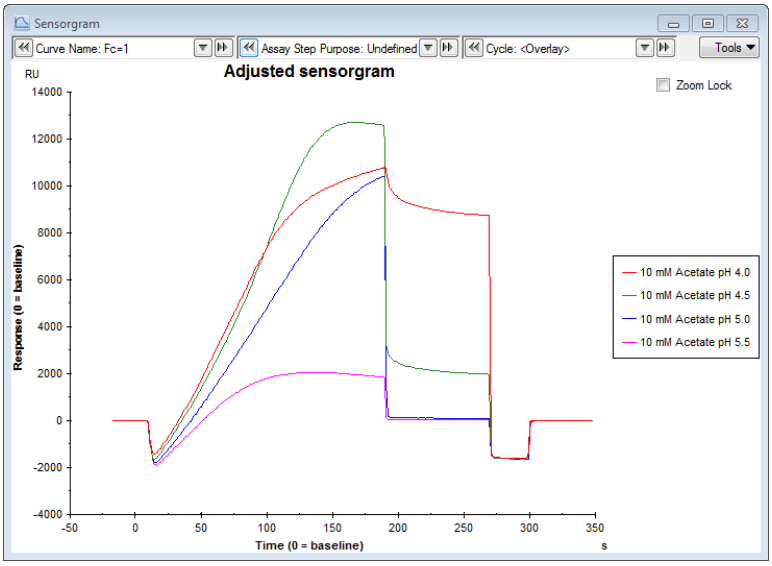
Follow the steps below to run a pH scouting run.

Step	Action
1	Prepare the instrument with an unused sensor chip.
2	Open a method template for pH scouting from the start screen. The predefined Biacore template (in the folder ***Surface Preparation***) is recommended.
3	Check through the template and customize settings to suit your needs if required (see *Chapter 5 Methods, on page 42*).
4	Click ***Setup Run***.
5	Choose the ***Flow path***. pH scouting is run in a single flow cell with no reference. Click ***Next***.
6	Complete the sample table with samples and buffer names as required. The predefined template is set up for one sample tested with the 4 standard acetate buffers available from Cytiva.
7	Continue to set up the run as directed in the remainder of the template (see *Section 5.8 Setup Run, on page 65*), then start the run.

Evaluating pH scouting

pH scouting is best evaluated from a sensorgram item in the Evaluation Software (see *Section 8.2 Sensorgram items, on page 100*). Follow the steps below to set up the item for evaluation.

Step	Action
1	Open the pH scouting run in the Evaluation Software.
2	Click ***Sensorgram*** in the ***Inspection*** group. *Result:* A new ***Sensorgram*** item is created with the sensorgrams in an overlay plot.
3	Check that the correct flow cell is displayed (there will normally only be one flow cell available).

Step	Action
	Choose ***Tools*** →***Sensorgram Adjustment*** and apply the following adjustments: • ***X-Adjustment***: Zero at report point ***baseline***. • ***Y-Adjustment***: Zero at report point ***baseline***.
4	Choose ***Tools*** →***Color by*** →***Buffer_name***. Result: The sensorgrams are identified according to the buffer used.
	
5	Judge the optimum buffer pH on the basis of the binding behavior: at pH suitable for immobilization, the ligand should bind rapidly to the surface during the injection and dissociate completely after the end of the injection. The optimum is generally the highest pH value (i.e. the mildest condition) that gives sufficient ligand binding, not necessarily the value that gives the highest ligand binding.

4.2 Performing immobilization

Introduction

The ***Immobilization*** tool supports immobilization of ligand in any combination of the four flow cells in one run. Immobilization may be performed independently in a separate cycle for each flow cell, in flow cell pairs (Fc 1-2 and 3-4), or in all four flow cells in one cycle. See the Biacore Sensor Surface Handbook for more information about ligand immobilization.

Note: *Performing immobilization in multiple flow cells in a single cycle does not guarantee identical immobilization levels in each flow cell. In general, immobilization yields tend to be lower in flow cells later in the flow path.*

Procedure

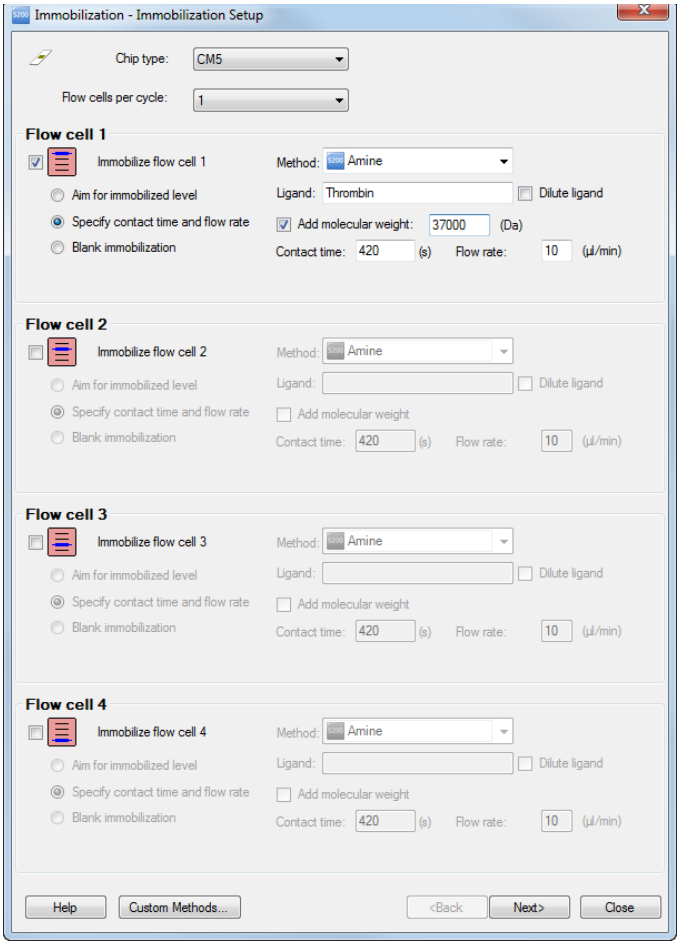
Follow the steps below to set up an immobilization run.

Step	Action
1	Open the Biacore template ***Surface preparation*** →***Immobilization***.

Step	Action

Result:

The ***Immobilization Setup*** dialog opens.



2 Choose the ***Chip type*** for which the procedure will be used. This will determine the predefined methods that are available for immobilization. The currently docked chip type is chosen by default: if you change the chip type you will be able to create and save an immobilization template, but you must dock the corresponding chip type before the immobilization can be performed.

3 Choose the number of ***Flow cells per cycle*** and check the flow cells where you want to perform immobilization.

Step	Action
4	For each flow cell or group of flow cells, set the immobilization parameters as follows. • Choose the immobilization method. Predefined methods are provided for standard immobilization chemistries. Customized methods can be defined by clicking on the ***Custom Methods*** button (see *Section 4.3 Custom immobilization methods, on page 32*). Predefined methods are marked with an S200 icon ( ). • Enter the name of the ligand. • Check ***Add molecular weight*** and enter the ***Ligand MW*** if desired. This information is required in ***Binding Level Screen*** experiments for calculating theoretical R_{max} levels for the ***R>Rmax*** binding behavior indicator (see *Binding behavior indicators, on page 138*). It can be omitted if the sensor chip will not be used for experiments of this kind. • Check ***Dilute ligand*** to dilute the ligand from stock solution into immobilization buffer in the autosampler immediately before injection. Use this option if the ligand is not stable in immobilization buffer. • Choose how immobilization will be controlled (see *Immobilization control options, on page 30* for options).
5	Click ***Next*** to continue with the instrument preparation and run setup steps as described in *Section 5.8 Setup Run, on page 65*.

Immobilization control options

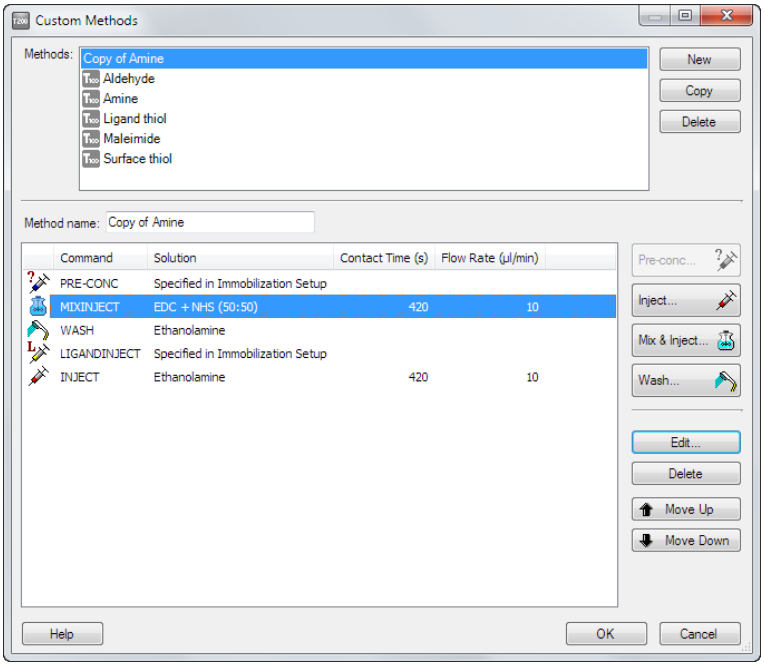
Alternatives for how the immobilization will be performed are listed in the following table.

Option	Description
Aim for immobilized level	Specify a target level. The immobilization procedure will attempt to reach this level as described below. This option is only available for immobilization in one flow cell per cycle. ***Aim for immobilized level*** injects 10 µl ligand solution at a flow rate of 5 µl/min, to estimate the rate of accumulation of ligand on the surface as a result of electrostatic preconcentration. The surface is washed to remove traces of ligand and then activated. The procedure then uses ligand contact times based on the preconcentration estimate to attempt to reach the specified target level. If preconcentration is either too fast or too slow to permit the target level to be reached, this will be reported and immobilization will not be performed. The preconcentration step is included in predefined methods for CM-series sensor chips but is optional in customized methods (see below). If ***Aim for immobilized level*** is chosen together with a custom method that does not include a preconcentration injection, the immobilization procedure will activate the surface and then inject short pulses of ligand until either the target level or the maximum total ligand volume of 150 µl is reached. This can be used to conserve valuable ligand without losing the benefits of aiming for a target immobilization level, and can be useful for sensor chips where preconcentration cannot be performed, such as Sensor Chip Protein A and Sensor Chip SA.
Specify contact time and flow rate	Immobilization will be performed using the contact time and flow rate for the ligand injection as specified. Enter the ligand contact time and flow rate.
Blank immobilization	The surface will be activated and deactivated in accordance with the immobilization method but no ligand will be injected. No additional information is required.

4.3 Custom immobilization methods

Defining custom immobilization methods

Click ***Custom Methods*** in the immobilization setup dialog to define customized immobilization methods.



Click ***New*** to create a new blank method. Select an existing method and click ***Copy*** to make a copy of the method or ***Delete*** to delete the method. You cannot delete the predefined methods (marked with an S200 icon ( ).

For a new method, enter a method name and construct the sequence of injections for the method using the buttons to the right of the main panel. The ligand injection is created automatically and cannot be deleted: solution and contact time for the ligand injection are specified in the main wizard dialog. A method may only contain one ligand injection. Other injections have the following functions:

Injection	Description
Pre-conc	Injects 10 µl of ligand solution at 5 µl/min to estimate the rate of preconcentration. This step is only performed if the option ***Aim for immobilized level*** is chosen when the immobilization method is used. A method may only contain one ***Pre-conc*** injection. The ***Pre-conc*** injection should always be placed before surface activation: it will usually be first in the method, although it may be preceded by a surface conditioning injection if required. If you place the ***Pre-conc*** injection after the surface activation, it will be executed there and the ligand will be immobilized on the activated surface. After the ***Pre-conc*** injection, the surface is washed with a solution specified in the immobilization setup dialog, to remove any ligand that may remain on the surface. Do not use a ***Pre-conc*** injection with Sensor Chip SA, since biotinylated ligand will bind to the surface and cannot be removed.
Inject	Injects a specified solution with a specified contact time and flow rate. Details are entered in the dialog box that appears when you click ***Inject***.
Mix & Inject	Mixes two specified solutions and performs an injection of the mixture. Details are entered in the dialog box that appears when you click ***Mix & Inject***.
Wash	Washes the flow system (by-passing the sensor surface). Details are entered in the dialog box that appears when you click ***Wash***.

Select an injection and use the ***Edit***, ***Delete***, ***Move up*** and ***Move down*** buttons to edit the injection details, remove the injection from the method and change the order of injections in the method.

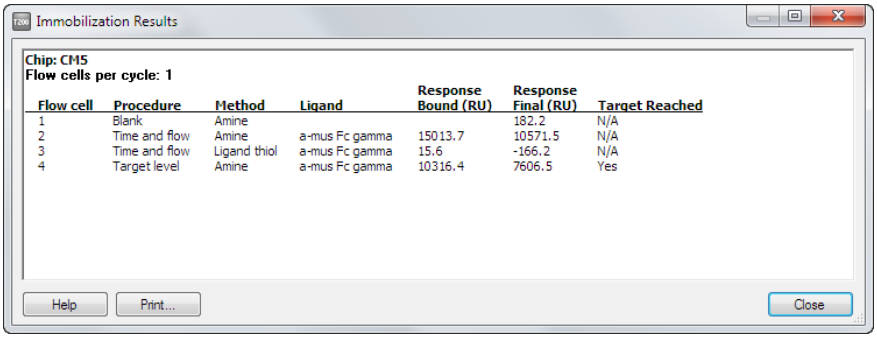
Custom methods are stored in the immobilization template. If you need the same method in more than one template, save a copy of the template.

4.4 Immobilization results

Presentation of results

The results of an immobilization run are summarized in the Control Software and logged in the ***Chip Properties*** when the run is completed. To view the immobilization details for the chip currently docked in the instrument, choose ***View*** →***Chip Properties*** from the main menu.

The same information can be accessed under ***File*** →***Properties*** in the Evaluation Software.

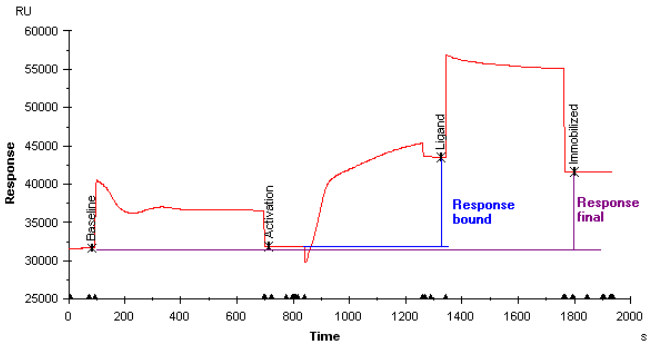


The summary lists the procedure and method, the name of the ligand, and whether the target was reached with ***Aim for immobilized level***.

Result files from immobilization can also be opened in the Evaluation Software if you want to prepare other sensorgram displays or plots.

Response levels

Two response values are reported as illustrated below, one directly after the ligand immobilization and one after the deactivation injection. The difference between these values is an indication of the amount of non-covalently bound ligand that is washed from the surface by the deactivation injection.



Note: *The **Response bound** value does not include the contribution from activation by EDC/NHS. For low ligand levels, this value can give a better indication of the amount of ligand immobilized.*

4.5 Interaction characteristics

Testing interaction characteristics

Efficient design of interaction analysis experiments often requires a general knowledge of the interaction characteristics - appropriate analyte concentrations and injection times, analyte binding capacity of the surface, and so on. The predefined ***Interaction characteristics*** template in the ***Assay development*** folder is designed to test these characteristics for a new interaction.

The template is set up as a single-cycle kinetics injection (see *Appendix C.1 Experimental formats, on page 205*) with five sample concentrations covering a 100-fold concentration range. Use the suggested concentrations if the interaction is completely uncharacterized, or adjust the concentrations appropriately if you have some knowledge of the characteristics. You may need to run the test more than once if the initial settings turn out to be unsuitable.

Evaluating interaction characteristics

Inspect the sensorgrams to evaluate interaction characteristics experiments. In fortunate cases, where the sensorgrams show concentration-dependent binding with kinetic characteristics in each injection, it may be possible to get a preliminary estimate of kinetic and affinity constants for the interaction and binding capacity (R_{max}) of the surface (see *Chapter 11 Evaluating kinetics and affinity, on page 142*). Note however that this will only be a preliminary estimate, since the requirements for careful kinetic and affinity analysis are not fulfilled (in particular subtraction of blank sensorgrams).

4.6 Buffer scouting

Buffer scouting options

The choice of running buffer can have a significant effect on interaction characteristics as well as aspects of assay performance such as non-specific binding to the sensor surface. Two approaches for buffer scouting are supported in Biacore S200 Control Software. Predefined Biacore methods are provided for each approach.

Approach	Description
Using the buffer selector	By using the buffer selector, up to four different running buffers can be tested in the same run. The buffer is chosen by assay step in the method (see *Creating and editing assay steps, on page 49*). A Biacore method template for testing four buffers is provided with the software. Each time the running buffer is changed, a startup cycle is run to stabilize the system.
Using ABA inject	The A-B-A injection type sandwiches a sample (B) between two buffer plugs (see *Sample injection, on page 59*), allowing the interaction to be tested in different buffers without changing the running buffer. ***Note:*** *This approach does not support startup cycles to stabilize the system after a buffer change.*

Running buffer scouting with the buffer selector

Follow the steps below to set up a buffer scouting run using the buffer selector.

Step	Action
1	Prepare the instrument with an appropriate sensor chip.
2	Open a method template for buffer scouting using the buffer selector. The predefined Biacore template in the folder ***Assay development*** is recommended.
3	Check through the template and customize settings to suit your needs if required (see *Chapter 5 Methods, on page 42*). The predefined template is set up to use four buffers.
4	Click ***Setup Run***.
5	Choose the ***Flow path***. A reference-subtracted flow path (***2-1*** or ***4-3***) is recommended. Click ***Next***.

Step	Action
6	Complete the sample table for each assay step with samples as required. Use the respective buffer as sample in the ***Startup*** assay steps. The predefined template is set up for three samples in each buffer.
7	Continue to set up the run as directed in the remainder of the template (see *Section 5.8 Setup Run, on page 65*), then start the run.

Running buffer scouting with *A-B-A* injection type

Follow the steps below to set up a buffer scouting run using the ***A-B-A*** injection type.

Step	Action
1	Prepare the instrument with an appropriate sensor chip.
2	Open a method template for buffer scouting using the ***A-B-A*** injection type. The predefined Biacore template in the folder ***Assay development*** is recommended.
3	Check through the template and customize settings to suit your needs if required (see *Chapter 5 Methods, on page 42*).
4	Click ***Setup Run***.
5	Choose the ***Flow path***. A reference-subtracted flow path (***2-1*** or ***4-3***) is recommended. Click ***Next***.
6	Complete the sample table for the ***Sample*** assay step with samples and flanking solutions as required. Use the respective buffer as flanking solution, and prepare samples in each buffer. The predefined template is set up for three samples in four buffers.
7	Continue to set up the run as directed in the remainder of the template (see *Section 5.8 Setup Run, on page 65*), then start the run.

Evaluating buffer scouting

Evaluate buffer scouting runs using sensorgram and plot items as appropriate for the properties that are expected to be influenced by buffer variation (for example, binding rates, binding levels, non-specific binding to the reference surface and so on). The results are evaluated in the same way for buffer scouting using the buffer selector and the ***A-B-A*** injection type.

Use the ***Assay Step Purpose*** selector in sensorgram or plot items to exclude startup cycles from the display. Use ***Tools*** →***Color by*** with the appropriate parameters to help identify the different curves.

If you have run buffer scouting for several samples in the same run, prepare a separate evaluation item for each sample, using the ***Cycle selector*** to choose the cycles for each sample.

4.7 Regeneration scouting

Description

Regeneration involves removal of non-covalently bound material from the sensor surface after sample injection in preparation for the next sample. Regeneration scouting is necessary if regeneration conditions are not known, and may be used to confirm the performance for assays where regeneration conditions are known (such as capture assays where regeneration removes both ligand and analyte).

A predefined ***Regeneration scouting*** template is provided in the ***Assay development*** folder. The template runs five sample and regeneration cycles for each condition tested. Since the effect of regeneration in one cycle is seen in the following cycle (see *Evaluating regeneration scouting, on page 41*), the template also includes an ***Extra Sample*** assay step with one cycle that repeats the last regeneration condition tested.

Testing regeneration conditions

Parameters that can be tested in regeneration scouting are regeneration solution, contact time and flow rate. These can be set as variables in the ***Regeneration*** injection. You are strongly recommended to vary only one of these parameters at once to simplify interpretation of the results. For this reason, the pre-defined template is set up with only the regeneration solution set as a variable (the solution is normally the first parameter you should test). Change the variable settings in the cycle type definition if you want to test the other parameters (see *Variables, on page 54*). Start scouting with the mildest conditions to reduce the risk of damaging the ligand with harsher conditions early in the run.

Running regeneration scouting

Follow the steps below to set up a regeneration scouting experiment.

Step	Action
1	Prepare the instrument with an appropriate sensor chip.
2	Open a method template for regeneration scouting. The predefined Biacore template in the folder ***Assay development*** is recommended.
3	Check through the template and customize settings to suit your needs if required (see *Chapter 5 Methods, on page 42*). In particular, make sure that the desired parameter is set as variable in for the ***Regeneration*** injection.
4	Click ***Setup Run***.
5	Choose the ***Flow path***. Regeneration scouting is run in a single flow cell with no reference. Click ***Next***.
6	Complete the sample table with values for the variable parameter.

Step	Action
7	Continue to set up the run as directed in the remainder of the template (see *Section 5.8 Setup Run, on page 65*), then start the run.

Evaluating regeneration scouting

Evaluation of regeneration scouting is based on two parameters:

- The absolute baseline response reflects the regeneration efficiency (an increase in baseline may reflect inadequate regeneration, a decrease may reflect loss of ligand from the surface).
- The relative binding response for sample shows how well the binding capacity is retained (at least for the analyte concentration used).

Set up separate QC plots for the report points ***baseline*** (absolute response) and ***binding*** (relative response). Use ***Tools*** →***Color by*** to help distinguish the regernation conditions. Remember that the effect of regeneration in one cycle is seen in the next cycle: for example, if you have run 5 replicate cycles of several conditions, the results for the first condition are seen in cycles 2-6, the second in 7-11 and so on.

5 Methods

About this chapter

This chapter describes how to create and edit method templates.

In this chapter

Section		See page
5.1	Introduction	43
5.2	Method overview	45
5.3	General settings	46
5.4	Assay steps	49
5.5	Cycle types	53
5.6	Injection options	59
5.7	Verification	64
5.8	Setup Run	65

5.1 Introduction

Method structure

Methods are defined in a series of sections representing different aspects of the method definition.

Section	Description
Overview	The overview summarizes the method definition. This is displayed on the right of the workspace in all method sections.
General settings	Here you define general parameters such as the concentration unit for samples, sample compartment temperature, data collection rate, detection mode and buffer names.
Assay steps	Assay steps represent distinct parts of the assay, which may be run once or repeated during the course of the assay. Examples of assay steps are start-up, solvent correction, sample analysis or control sample analysis. Assay steps are assigned an ***Assay step purpose***, which is used for identifying data in the Evaluation Software, and are connected to ***Cycle types***, which define the sequence of operations performed in the assay step.
Cycle types	Cycle types define the details of how assay steps will be performed, in terms of sample and reagent injections. The same cycle type can be used in different assay steps. For example, assay steps for samples and control samples will typically use the same cycle type, ensuring that controls are analyzed in exactly the same way as samples. Parameters in a cycle type definition may be variable, so that they can be assigned a series of different values when the method is used. Sample names will typically be variable. The number of values for variable parameters together with assay step repetition determines the number of cycles in the run. Report points can also be defined for each cycle type.

Section	Description
Verification	Once the method has been defined in full, this section verifies that all aspects are consistent and completely specified. The verification results are reported in the work area. A method that does not pass verification can be saved but cannot be run. ***Note:*** *Verification only checks the consistency and completeness of the method. It does not in any way verify that the method is suitable for the intended purpose.*

The sections are described in detail in the following sections.

You can save a method template at any time, even if it has not been completed. The template must however be completed before it is used to control a run.

Method templates

Method definitions may be saved as *templates* for later re-use and modification. Templates may contain complete (ready to run) or partial method definitions. Partial templates must be completed before they can be run.

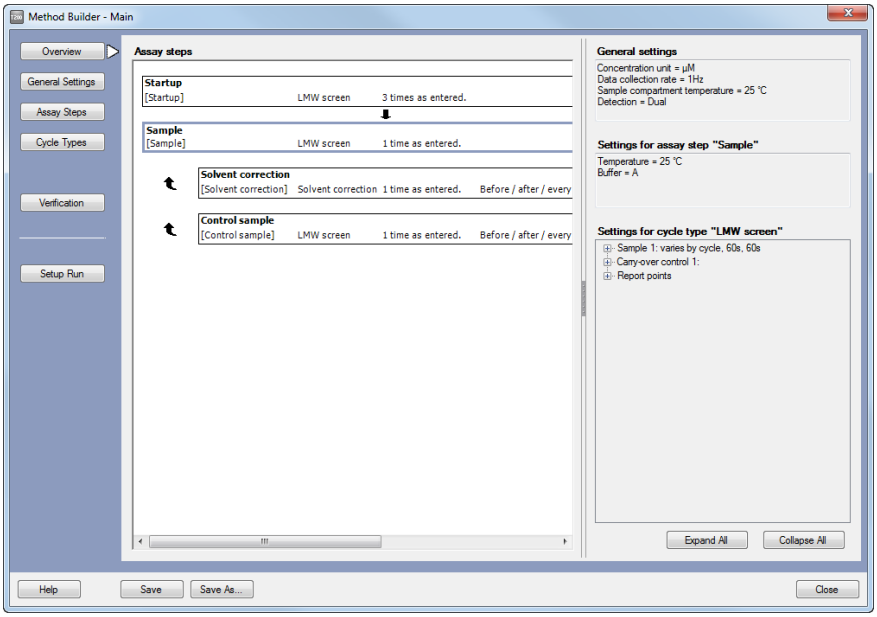
A set of predefined ***Biacore Methods*** for common applications is provided with the software (see *Appendix B Organization of Biacore templates, on page 201* for a description of how the templates are organized). These define the essential settings for the respective application, forming an easy starting-point for setting up your own applications. The predefined method templates themselves cannot be changed, but they may be edited and saved with a new name.

Method templates are saved by default in a methods folder, defined under ***Tools →Preferences*** (see *Section 2.2 Software preferences, on page 13*). The file type is ***.Method***. Details of the method are also saved in the result file from every method run, so that the method settings used for the actual run are documented even if the method template file is edited or deleted.

Double-click a template or select a template in the start screen (see *Templates, on page 9*) and click ***Open*** to open the template. To start with a blank template, choose ***Blank Template***.

5.2 Method overview

Presentation

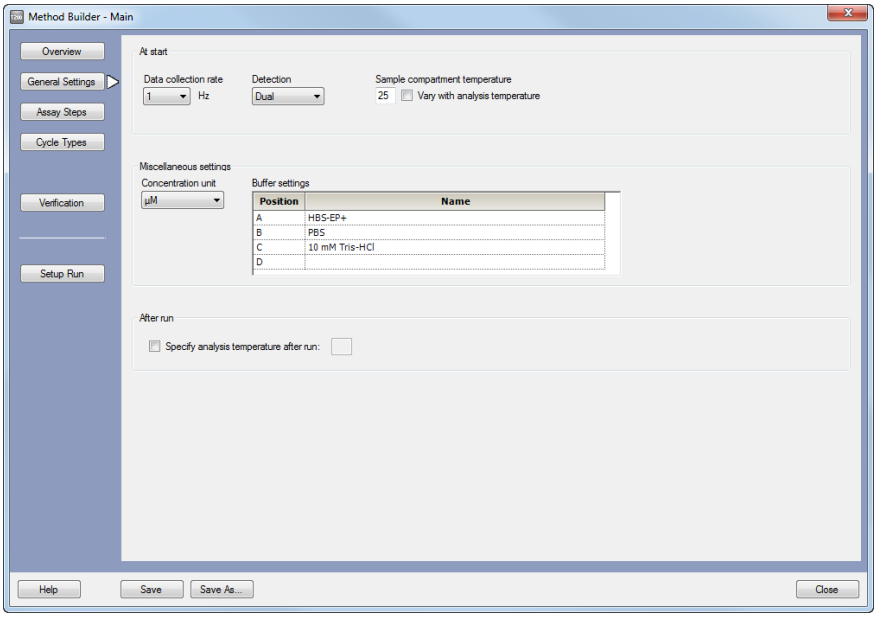


The method overview provides a summary of the method. The main panel shows the assay steps in the method (see *Section 5.4 Assay steps, on page 49*). Click on an assay step to show the settings for the step and the details of the cycle definition (see *Section 5.5 Cycle types, on page 53*) in the panels to the right. The cycle definition is listed as a series of injections: to see injection details, expand individual injections by clicking on the **+**-marking or use the ***Expand All*** button to expand all injections in the panel.

Settings cannot be changed in the overview display.

5.3 General settings

Presentation



Settings specified here will apply throughout the run.

Data collection rate

Choose the data collection rate (expressed as data points per second). Higher settings will provide better resolution for kinetic analysis of fast interactions, but will result in larger result files.

The highest data collection rate should only be used for analysis of very fast interactions. Using the highest data collection rate for interactions that require relatively long injection and dissociation times (more than about 1 minute) will only increase result file size and demand greater computing capacity without improving the results.

Detection

This setting determines how data is recorded from the flow cells:

Setting	Description
Single	Records data from one flow cell according to the flow path chosen when the run is set up. Data is not recorded from the other flow cells.

Setting	Description
Dual	Records data from one flow cell pair (1,2 or 3,4) according to the flow path chosen when the run is set up. Data is not recorded from the other flow cell pair.
Multi	Records data from all four flow cells regardless of the flow path.

The setting affects the choice of flow path that can be made for each injection in the cycle type definitions (see *Section 5.5 Cycle types, on page 53*), and the flow path for the overall run in ***Setup Run*** (see *Section 5.8 Setup Run, on page 65*). Use the setting ***Multi*** if you are not sure what you need: this will provide maximum flexibility for the flow path settings.

Note: *If you set detection to **Multi** and then inject sample over fewer than all four flow cells, buffer will be stationary and data recorded in the flow cells that are not used will be meaningless.*

Using flow cell pairs

The flow cells are designed to be used in pairs for optimal performance (Fc1-2 and Fc3-4). If both pairs are used separately in the same run, bear in mind that buffer before and after the sample analysis passes over all four flow cells, so that any material released from Fc1-2 will be carried downstream to Fc3-4. As a general rule, use Fc3-4 first so that the analysis is not contaminated by material from Fc1-2.

Sample compartment temperature

This is the temperature in the sample compartment (not the analysis temperature at the flow cell, which is set for each assay step). Check ***Vary with analysis temperature*** to set the sample compartment temperature automatically to the same value as the analysis temperature.

Concentration unit

This setting defines the unit for entering sample concentrations. The unit must be specified here, and cannot be changed at any other step in the assay definition. The unit can however be changed in the Evaluation Software when the results of the run are evaluated.

Buffer settings

Enter names if desired for the buffers in bottles **A** through **D**. Names entered here will be displayed in the ***Prepare Run Protocol*** during run setup. Different buffers may be chosen for different assay steps (see *Section 5.4 Assay steps, on page 49*).

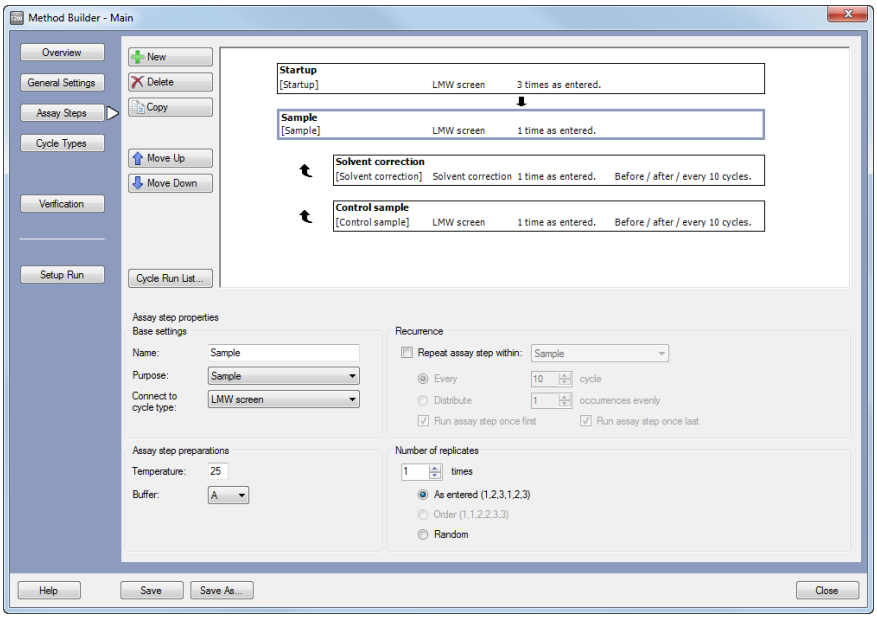
Specify analysis temperature after run

Check this option and enter a temperature to set the analysis temperature when the run is completed. The rack temperature will also be reset if ***Vary with analysis temperature*** is checked. This setting provides automated control of the chip and detector environment after the completion of a run, for example in preparation for another run at a different temperature.

5.4 Assay steps

Introduction

This section determines the main structure of the method in terms of assay steps. Steps at the top level are executed in the order given. Nested steps (indented and marked with the ↳ symbol) are executed within the context of the step in which they are nested, as specified by the settings for ***Recurrence*** (for example, the ***Solvent correction*** and ***Control sample*** steps in the illustration below will be repeated within the context of the ***Sample*** step).



Creating and editing assay steps

Follow the steps below to create a new assay step. To edit an existing assay step, select the step and edit the settings as required.

Step	Action
1	Click ***New***. A new step will be created with the name ***Assay step n*** at the end of the method. Alternatively, select an existing step and click ***Copy***, then edit the settings of the copied step. ***Note:*** *A copy of a top-level step will be placed at the end of the method. A copy of a nested step will be placed at the end of the nested level.*

Step	Action
2	Use the ***Move Up/Down*** buttons to move the new step to the required position in the method.
3	Change the assay step name to something more appropriate if desired. Each assay step in the method must have a unique name.
4	Assign a purpose to the assay step (see *Assay step purposes, on page 50*). The purpose will determine how data from the assay step is handled in the evaluation software.
5	Connect the assay step to a suitable cycle type. The cycle type must already have been created in the method (see *Creating and editing cycle types, on page 53*), but the settings can be defined or edited at any time.
6	Set the analysis temperature. Different temperatures may be used for different assay steps if required. The setting will also control the sample compartment temperature if the appropriate option is checked under *Section 5.3 General settings, on page 46*. If the actual temperature at the start of an assay step does not match the setting for the step, the system will wait until the set temperature is reached.
7	Select the running buffer to be used for the assay step. The default buffer is ***A*** (corresponding to buffer bottle and tubing **A** on the instrument).
8	If you want the assay step to recur within the context of another step, check ***Repeat assay step within*** and choose the step in which recurrence should occur. Options for repeated assay steps are described in *Options for assay step recurrence and replicates, on page 51*.
9	Set the number of replicates and replicate cycle order if the assay step is to be run more than once. Options for replicates are described in *Options for assay step recurrence and replicates, on page 51*.

Assay step purposes

An assay step may have one of the following purposes:

Purpose	Description
Calibration	Used for calibration curves in ***Affinity in solution*** assays (see *Chapter 13 Affinity in solution, on page 179*). This assay step should be connected to the same cycle type as the ***Sample*** step so that the calibration and sample analyses are performed in the same way.

Purpose	Description
Conditioning	Used to condition the sensor surface at the start of an assay. Conditioning at the beginning of a method is only required for sensor chips that do not use covalently immobilized ligand or capturing molecule, such as Sensor Chip SA.
Control sample	Used for control samples. This assay step should be connected to the same cycle type as the ***Sample*** step so that the control sample and sample analyses are performed in the same way, and should typically be repeated at intervals throughout the assay.
Sample	Used for sample analysis in all applications.
Solvent correction	Used for solvent correction cycles. This step should be connected to a cycle type designed for solvent correction, and should typically be repeated at intervals throughout the assay.
Startup	Used to condition the flow system at the start of an assay. This assay step will commonly be connected to the same cycle type as the ***Sample*** step.
Undefined	Used for assay steps that do not fit the predefined purposes. Assay steps with ***Undefined*** purpose will not be included in application-specific evaluation.

Note: *For many methods, the assay step name and purpose may often be the same (e.g.* ***Solvent Correction***, ***Sample***, ***Control Sample*** *etc). It is however important to remember that the name is intended for documentation from the user's perspective and may be chosen freely, while the purpose has significance for the step properties and for evaluation of the run and must be chosen from the predefined list.*

Options for assay step recurrence and replicates

For recurrent steps, set the options as described in the following table.

Option	Description
Every ... cycles	The step will recur at a fixed interval. The number of recurrences will depend on the number of cycles in the higher level step.
Distribute ... occurrences evenly	The step will recur a fixed number of times. The interval between recurrences will depend on the number of cycles in the higher level step.
Run assay step once/last	The assay step will be run at the beginning/end of the step higher level step.

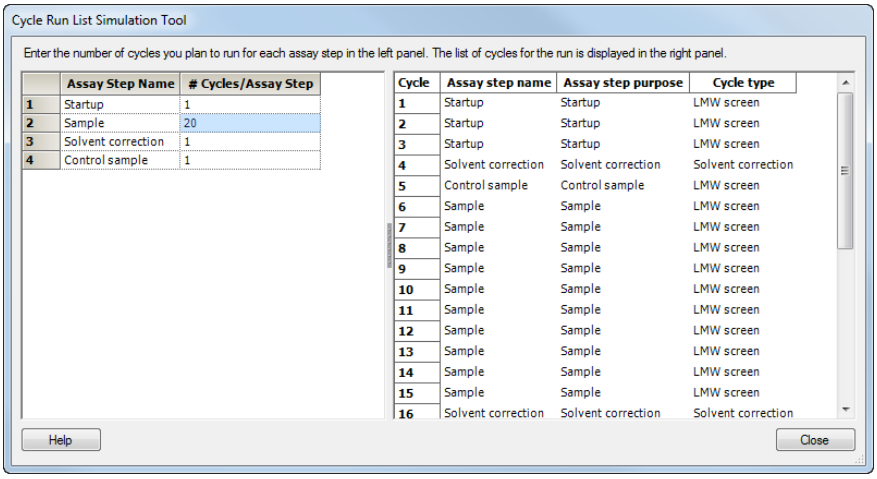
For replicate steps, set the order of replicates as described in the following table.

Option	Description
As entered	Performs all cycles in the step once, then repeats the step until the number of replicates is reached.
Order	Performs the first cycle in the step for the specified number of replicates, then the second cycle and so on.
Random	Randomizes the order of the cycles within the step until all cycles have been executed the specified number of times. The order is randomized each time the method is run.

If the step within which another step recurs is run in replicate, the recurring step is distributed among the total number of cycles including replicates.

Simulating the cycle order

Click the ***Cycle Run List*** button to simulate the number and order of cycles that the method will require according to the number of samples. Use this feature to check that the method will be executed as intended.

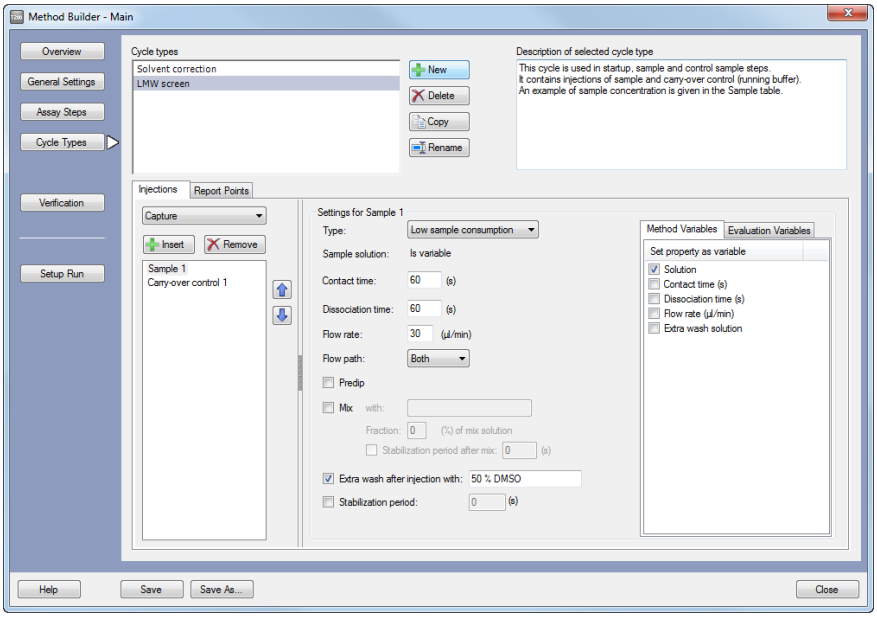


Enter the number of cycles to be run in each assay step in the left-hand panel. The simulated cycle list for the run as currently defined is shown in the right-hand panel.

5.5 Cycle types

Introduction

Cycle types define the detailed sequence of operations to be performed in a cycle, and are linked to one or more assay steps.



Note: *Cycle types are only active when they are connected to assay steps. A method template may contain cycle types that are not used.*

Creating and editing cycle types

Follow the steps below to create a new cycle type.

Step	Action
1	Click ***New***. A new cycle type will be created with the name ***Cycle_n***. Use ***Rename*** to change the name of the cycle type. Alternatively, select an existing cycle type and click ***Copy***, then edit the settings of the copied type.
2	Enter a free-text description for the cycle type if desired.
3	Set the sequence of injections on the ***Injections*** tab (see *Injections, on page 54*).

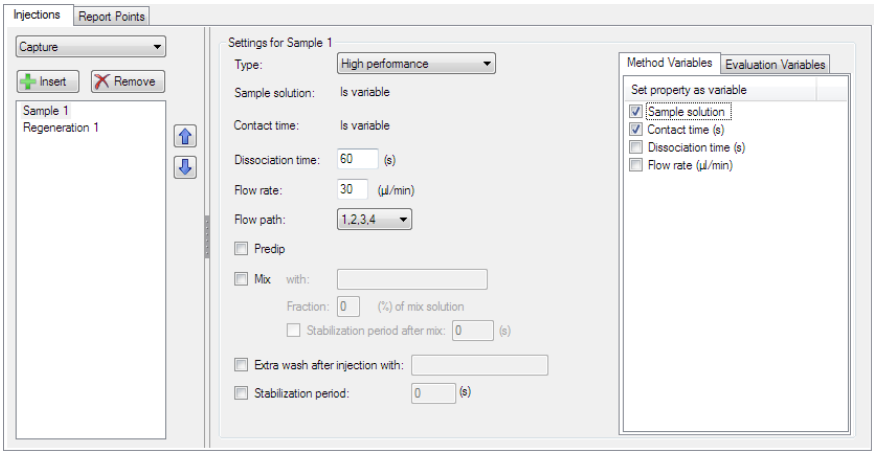
Step	Action
4	Define custom report points on the ***Report points*** tab if desired. See *Report points, on page 57*.

Injections

The injections in a cycle type define the operations that will be performed in the cycle.

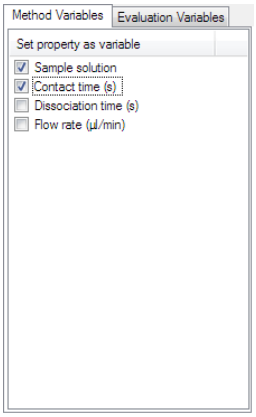
To add a new injection to the cycle definition, choose the injection type from the pull-down list and click ***Insert***. The injection will be inserted with default parameter settings immediately after the currently marked injection (or at the end of the cycle definition if no injection is marked). Change the settings as required. See InjectionDetails for details of the injection options.

Injections are executed in order from top to bottom. Use the up and down arrow buttons to move the injection to the required place in the sequence.

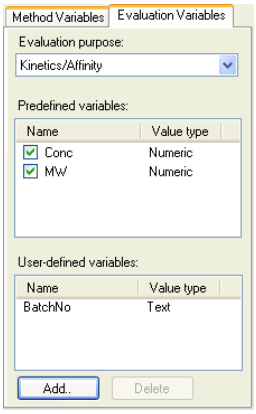


Variables

Parameters for many of the injections in a cycle definition may be set as variables. Values for variables are entered in the ***Setup Run*** step when the method is run (see *Section 5.8 Setup Run, on page 65*, and determine the number of cycles that will be performed in the run. Variables may be either *method variables* or *evaluation variables*.



Method variables



Evaluation variables

Method variables

Method variables such as sample name or contact time control the way the cycle will be performed. Parameters that may be set as method variables vary according to the type of injection.

Evaluation variables

Evaluation variables are used in evaluation of the data but do not affect the way the cycle is run. Evaluation variables may only be defined for ***Sample*** and ***General*** injections. Some evaluation variables are required for correct functioning of application-specific evaluation procedures (for example, kinetic evaluation requires a variable called ***Conc*** which holds the analyte concentration). These are selected from a predefined list specific to the purpose of the application. Other evaluation variables may be freely defined by the user, to hold information that is relevant to the assay but not required by an application-specific evaluation procedure (for example the sample batch number).

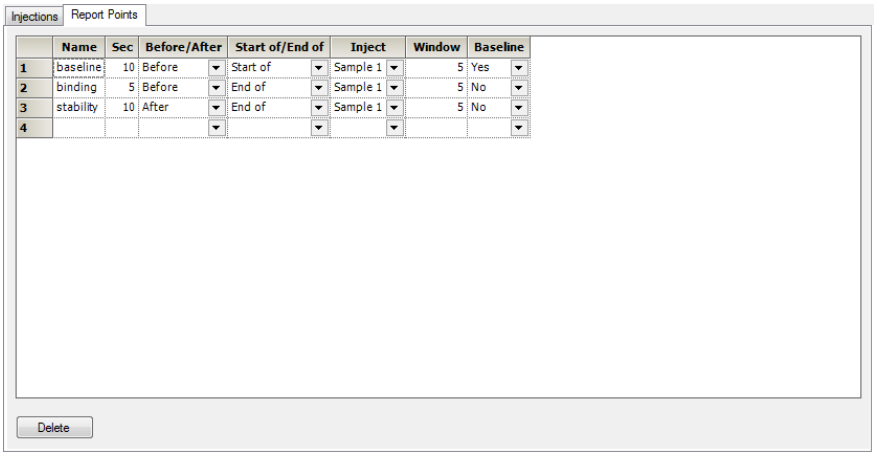
To set evaluation variables, first choose the evaluation purpose, then check the predefined variables you wish to use or add user-defined variables. User-defined variables are not linked to evaluation purpose.

Predefined evaluation variables for different assay purposes are described in the following table.

Purpose	Variables	Description
General Kinetics/Affinity Thermodynamics	***Conc***	Analyte concentration. Multi-cycle kinetics requires a single concentration variable. Single-cycle kinetics requires one concentration variable for each sample injection in the cycle.
	MW	Analyte molecular weight: required for molecular weight adjustment of report points, and for kinetic evaluation when the concentration is entered in weight-based units.
Kinetics (heterogeneous analyte)	***Conc1***, ***Conc2***	Analyte concentrations for the two analytes.
	MW1, ***MW2***	Molecular weight for the two analytes: these variables are required even if concentrations are entered in molar units, to determine the relative contributions of the two analytes to the observed response.
Affinity in solution[1]	***ConcB-calibration***	Concentration of interactant B used to construct a calibration curve.
	ConcB-fix	Concentration of interactant B in the sample mixture (the concentration of B is kept constant).
	ConcA-variable	Concentration of interactant A in the sample mixture (the concentration of A is varied).

[1] See *Experimental setup, on page 180* for details of how this assay is set up.

Report points



The ***Report points*** tab lists the report points in the cycle type definition, ordered as far as possible in the order they will appear in the cycle. Several injections have a predefined set of report points that are added to the list when the injection is included in the cycle type. You can add your own report points by filling in the details in the empty row at the bottom of the table. A new empty row is added whenever you create a report point.

Report points are set at defined times in relation to the start or end of injections in the cycle. Report points that are set outside the time range for the cycle (i.e. a significant time before the start of the first injection or after the end of the last injection) will not be created.

Note: *Do not position report points far away from events, so that they lose their relevance to the event, or so close to an event so that the report point window overlaps the event itself.*

Enter the report point settings as follows:

Setting	Description
Name	The report point name must be unique within the cycle type. Choose a name that reflects the function of the report point.
Sec	Enter the time in seconds between the report point and the event.
Before/ After	Choose whether the report point is to be placed before or after the event.
Start of/End of	Choose whether the report point is to be placed relative to the start or end of the injection.
Inject	Choose the injection to which the report point will be related.

Setting	Description
Window	Set the window for the report point calculation. The report point will be placed at the center of the window, and the reported response will be an average of the response values within the window. A window of 5 s is adequate for most purposes.
Baseline	Choose whether the report point will be defined as a baseline or not. Response values for report points that are not defined as a baseline will be calculated relative to the closest preceding baseline value.

5.6 Injection options

Fixed and variable parameters

Parameter values that are entered directly in the cycle type definition apply throughout the cycle. Some parameters are set as method variables: values for these are provided when the method is run. Sample names are typically variable so that the sample table is defined individually for each run.

Common injection settings

Several settings are common to a number of different injections. These are described in the table below.

Setting	Description
Predip	Check this option to dip the needle in a separate position before aspirating the solution to be injected. This will rinse the needle briefly to minimize carry-over effects from the previously injected solution. A separate predip position is created in the microplate or sample rack for each separate sample for which the cycle type is used.
Extra wash after injection	Check this option and specify a wash solution to perform an extra wash of the flow system after the injection. The flow system is washed automatically with buffer after each injection, but an extra wash with a different solution can be included if required. This wash solution does not pass over the sensor surface.
Stabilization period	Check this option and specify a time in seconds to introduce a delay before the next injection is started. This can sometimes be necessary (for example after regeneration steps) to allow the response to stabilize before performing the next injection.

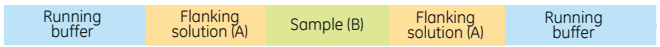
Capture injection

This injection is intended for injection of ligand over a capturing molecule at the beginning of a cycle. The injected solution, contact time and flow rate can be set as variables.

Sample injection

This injection is intended for injection of sample containing analyte. The injected solution, contact time, dissociation time and flow rate can be set as variables. Evaluation variables can also be defined for the ***Sample*** injection (see *Variables, on page 54*).

A ***Sample*** injection is identified by ***Type*** as listed in the following table.

Type	Description
High performance	Optimizes the injection for high performance in terms of rapid transition between buffer and sample at the beginning and end of the injection, at the expense of additional 25 µl sample consumption. Use this type for best resolution of fast binding kinetics.
Low sample consumption	Optimizes the injection for low sample consumption at the expense of slightly reduced performance. Low sample consumption injections are adequate for most applications except analysis of fast kinetics.
Single cycle kinetics	Injects a series of sample concentrations in the same cycle, intended specifically for single-cycle kinetics analysis (see *Appendix C.1 Experimental formats, on page 205*). The samples are injected in direct sequence, separated only by the time required to prepare the next injection. A dissociation time is included after the last sample injection.
Clean screen	Optimizes the injection for sample throughput and includes an adaptive wash after the injection. Specify the wash solution and the threshhold response value. The wash will only be performed if the response after the end of the injection is higher than the threshold.
Binding level screen	Optimizes the injection for sample throughput.
A-B-A	Injects sample ("***B***") flanked by a different solution ("***A***") which may be buffer (other than running buffer) or another sample. Use this option for example for competitive binding experiments or buffer scouting without changing running buffer. Contact times for flanking solution before and after the sample are set separately. The same flow rate is used throughout the injection. Dissociation times are not supported for A-B-A injections. 

The ***High performance*** and ***Low sample consumption*** options support a ***Mix*** function for mixing sample with a defined solution in the autosampler before injection. Check the ***Mix*** option and enter a mix solution and mixing fraction to use this function. Entering a fraction of e.g. 20% will mix one part of mixing solution with four parts of sample. The sample and mixing solution are taken from respective positions in the microplate or reagent rack and mixed in a third position. The option ***Stabilization period after mix*** allows you to specify a wait period between the mixing operation and injection of the mixed solution. Mixing solution and fraction may be set as variables.

Note: *Mixing is not supported in 384-well microplates.*

Enhancement injection

This injection is intended for injection of a secondary enhancement reagent following the sample injection. Enhancement reagents are most commonly used to amplify the analyte response and/or to confirm the identity of the bound analyte. The injected solution, contact time and flow rate can be set as variables.

Regeneration injection

This injection is intended for injection of a regeneration solution following the sample injection. Check ***High Viscosity Solution*** if the regeneration solution has a relative viscosity higher than about 3 (corresponding to about 35% glycerol or 40% ethylene glycol at 20°C). This will adapt the solution aspiration and injection procedure to handle the higher viscosity solution. The injected solution, contact time and flow rate can be set as variables.

Carry-over control injection

This injection injects a 30-second pulse of buffer over the surface at a flow rate of 40 µl/min, in order to check that there is no carry-over of analyte or other material from an injection earlier in the cycle. The injection is suitably placed at the end of the cycle, and can be used in a conditional context (see *Conditional injections, on page 62*) to perform additional buffer injections or regeneration steps if carry-over is detected.

Solvent correction injection

This injection injects a 20-second pulse of solvent correction solution over the surface at a flow rate of 30 µl/min. A solvent correction cycle should contain 4 to 8 ***Solvent correction*** injections for the different solvent concentrations used to construct the correction curve (see *Section 7.3 Solvent correction, on page 92*). Cycles containing solvent correction injections should only be used in assay steps with purpose ***Solvent correction***.

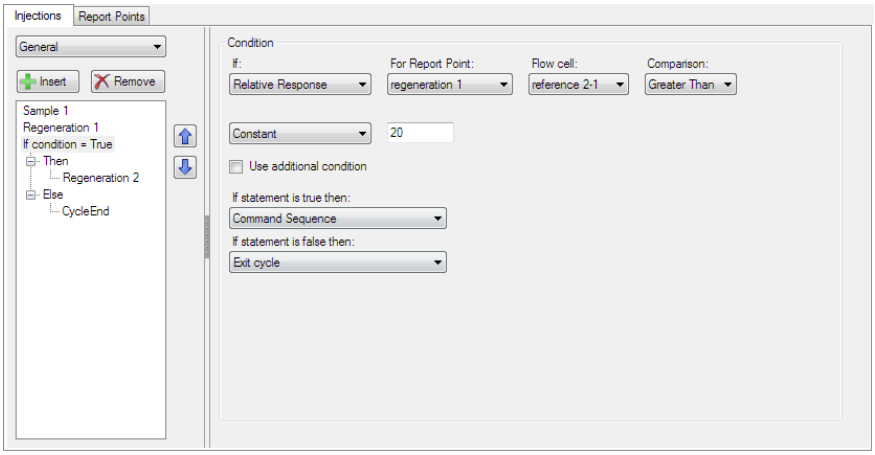
Do not mix ***Solvent correction*** injections with other injections in the same cycle type.

General injection

This injection is a general-purpose injection that supports the types ***High performance*** and ***Low sample consumption*** (see *Sample injection, on page 59*). ***General*** injections are not recognized as sample injections for evaluation of kinetics or affinity. The injected solution, contact time and flow rate can be set as variables. Evaluation variables can also be defined for ***General*** injections (see *Variables, on page 54*).

Conditional injections

Conditional injections are executed or skipped depending on the outcome of certain conditions. The illustration below shows a cycle which will perform an additional regeneration if the relative response after the first regeneration exceeds a specified value:



Follow the steps below to set up a conditional injection.

Step	Action
1	Insert an ***If...then*** injection at the appropriate place in the cycle definition.
2	Specify the condition. This is defined as the outcome of a comparison between a report point value (absolute response, relative response or slope) and a constant or another report point value with an added or subtracted constant value. Only report points that have already been set in the cycle definition may be used in the condition. Check ***Use additional condition*** to combine two conditions, using the logical operator ***AND*** (both conditions must be fulfilled) or ***OR*** (fulfillment of one condition is sufficient). The available comparison conditions are ***Greater than*** and ***Less than***. The condition ***Equal to*** is not supported since exact equality is an unpredictable condition in report point data. To construct an equality condition, combine a ***Greater than*** and a ***Less than*** condition so that a window of tolerance is created. For example, the combined condition "A greater than B-1 AND A less than B+1" is equivalent to A equals B with a tolerance of ±1.
3	Choose the actions to be taken when the condition is met and when it is not met. You may choose to execute an injection sequence, terminate the cycle or the method, or introduce a stabilization period.

Step	Action
	If you choose an injection sequence for either the ***True*** or ***False*** outcome, click on the appropriate branch of the conditional construction (***Then*** or ***Else*** respectively) and insert the injections you wish to be executed. If you leave the branch empty, the cycle will simply continue with the next injection following the ***If...then*** construction. You can use the ***Move up*** and ***Move down*** buttons to rearrange the order of injections within a branch, but you cannot move injections outside the branch in which they are placed. You cannot change the action if injections have been entered in the conditional injection sequence. Delete the injections first.

5.7 Verification

Purpose

This step checks that the method is correctly and completely defined. A method that does not pass the verification step can be saved but cannot be run. Verification may fail because parameters are missing (e.g. variables that are specified in the method have not been assigned values) or because the method construction is invalid (e.g. an assay step is not connected to a cycle type).

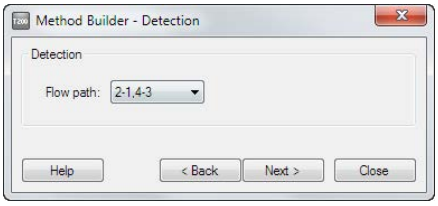
Note: *The verification step only examines the method construction, and does not check whether the method fulfils the requirements for assay-specific evaluation.*

5.8 Setup Run

Procedure

Follow the steps below to set up a run from the method template. All settings including any sample information may be saved in the method template. All settings are also saved in the result file.

Step	Action
1	Set the flow path for the method in the ***Detection*** dialog.

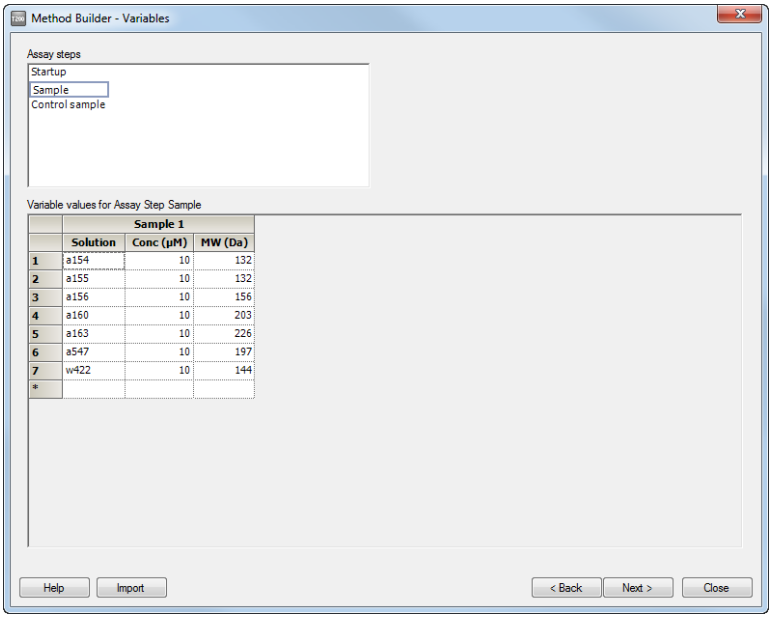


You can only choose a flow path that is consistent with the ***Detection*** setting for the method (see *Detection, on page 46*).

2	Assign values to variables (typically a sample table, see *Variables, on page 54*). Each row in the variables table corresponds to a cycle in the run. A new empty row (marked with an asterisk) is created automatically at the bottom of the table as soon as data is entered. Columns in the table correspond to variables for the cycle type used in the assay step, and are grouped according to the injections in the cycle type definition.

Step	Action

Depending on how the method is defined, there may be variable tables for several assay steps. Method variable values must be entered in all tables before you can continue to the next step. Evaluation variables may be left blank if desired at this step and values entered in the Evaluation Software.

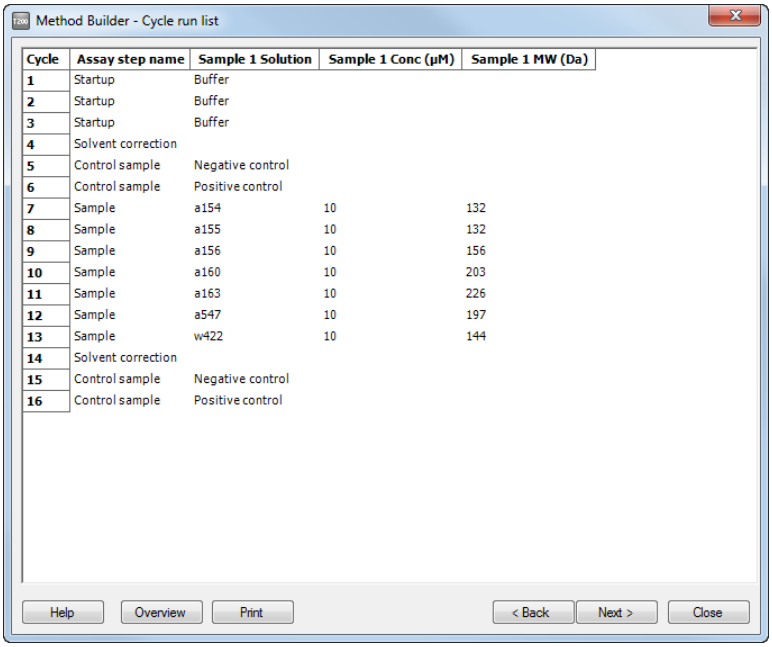


Use the right mouse button in the variables table to access functions for copying and pasting cell contents and for inserting and removing rows. The columns in the table are listed in the order they are defined in the method.

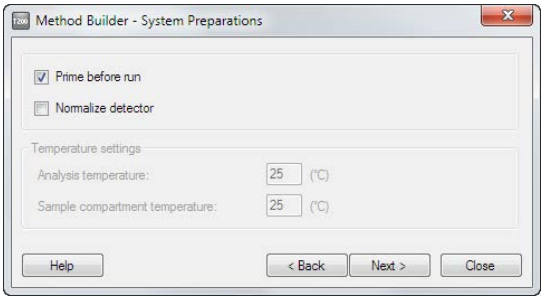
Click ***Import*** to import the variable values from an external file. See *Sample information import, on page 198* for details of the import format.

3 Click ***Next*** to view a complete summary of the cycles that will be performed in the run. This view is for information only and cannot be edited. Check through the cycle list to confirm that the run will proceed as intended. Click ***Overview*** to display the method overview (see *Section 5.2 Method overview, on page 45*) if you need to check the cycle run list against the method definition.

Step **Action**



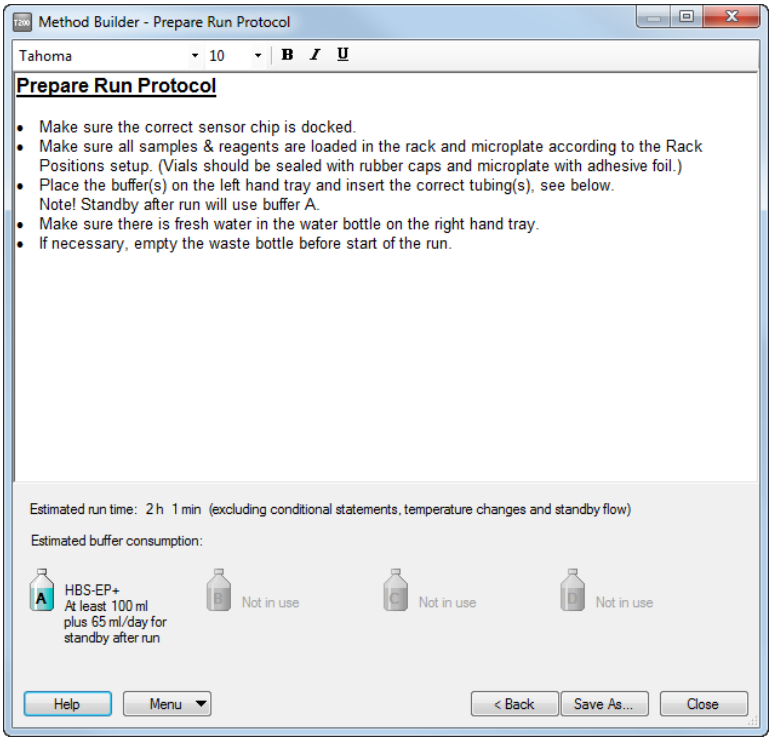
4 Choose whether to include ***Prime*** and ***Normalize*** at the beginning of the run. See *Tools, on page 10* for recommendations.



- For immobilization runs, set the analysis temperature and sample compartment temperature.
- For all other types of run, the temperature is specified in the method, and the options are not available in this dialog.

5 The ***Rack positions*** step shows where samples and reagents are to be placed in the microplate and/or rack. Positions are color-coded by region according to sample and reagent categories (see *Automatic positioning, on page 71* For details of how to work with the Rack Positions dialog, see *Rack positions dialog, on page 69*.

Step	Action
6	The ***Prepare Run Protocol*** step provides instructions to the user when the run is started and displays the estimated time and buffer requirements for the run. A suggested general protocol is provided by default. Edit the text if required.



Note:

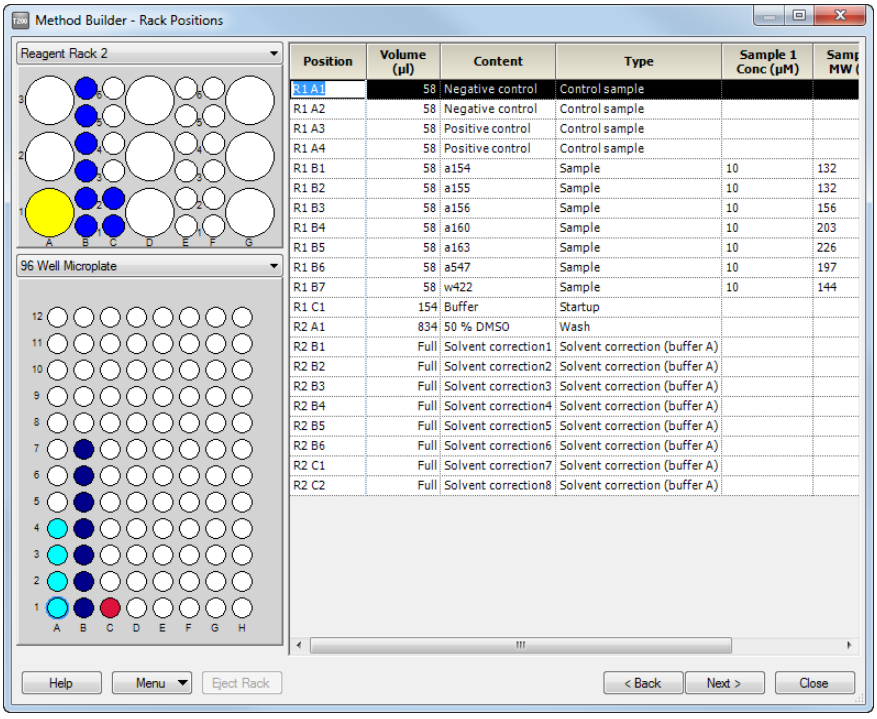
The estimated run time and buffer consumption are minimum values. Time for temperature equilibration and for adaptive operations such as immobilization with ***Aim for immobilized level****, adaptive wash and* ***If...then*** *constructions is not included.*

The estimated buffer requirement includes a dead volume of at least 50 ml in the buffer bottle and is rounded up to the nearest 100 ml, but does not include any consumption during standby after the run.

7	Click ***Start Run*** and provide a file name for the results to start the run, or click ***Save As*** to save the complete method.

Rack positions dialog

The ***Rack Positions*** dialog shows where samples and reagents are to be placed in the microplate and/or rack.



Managing rack positions

Use the pull-down lists above the respective illustrations to change the reagent rack and microplate types. If you change a rack or microplate type, all positions in the affected rack or plate will be cleared and must be reassigned either manually or automatically.

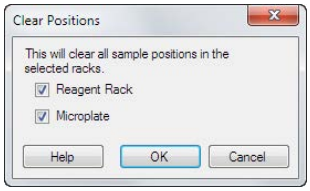
Hover with the cursor over a position to display a tool tip describing the position content. Empty positions show the position capacity and dead volume. Used positions show in addition the content name and the volume that will be used.

Note: *The volumes listed in the table are minimum volumes. Use slightly larger volumes if material is available to allow for variations in the dead volume in microplates and vials.*

You can change sample and reagent positions manually in two ways:

- Click on a sample or reagent in the sample plate and rack illustration and drag it to a new (empty) position. You cannot drag to a position that does not have sufficient capacity for the required volume of sample or reagent.
- Enter an unused position directly in the ***Position*** column in the table.

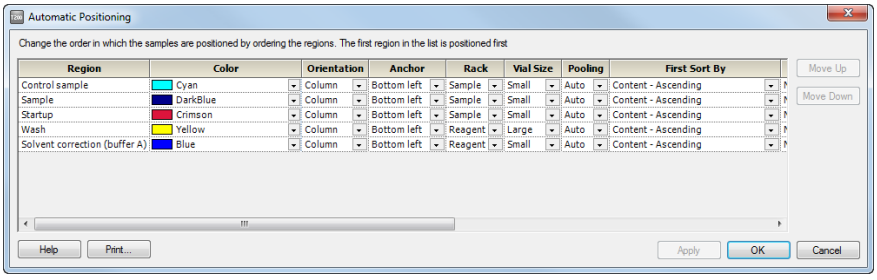
Use the ***Menu*** button to access additional functions for rack positioning as described in the following table. These functions are not available if the positions have been locked.

Function	Description
Clear Positions	This option clears the entries in the Positions column for the selected rack or plate.  Positions that are cleared must be reassigned before the run can be started. To reassign positions one by one, select a row in the positioning table and click on the required (empty) position in the illustration. To reassign all positions in one operation, choose ***Default Positions*** or ***Automatic positioning*** from the menu.
Default positions	This option restores all entries to default positioning. The default positioning is determined from the type and volume of solution in combination with the currently selected rack type. Choosing ***Default Positions*** overrides any changes that have been made in the rack positions, even if the changed positions have been saved in the method template.
Automatic positioning	This option controls the way samples and reagents are positioned automatically. See *Automatic positioning, on page 71* for details.
Save Method ***Save Method As***	Saves the method template, with either the same or a different file name.
Custom Position Import	This option imports positioning information from an external source. The option is only available if ***Enable custom position import*** is checked in ***Tools →Preferences*** (see *Section 2.2 Software preferences, on page 13*). Choosing the option first exports the rack positions table to a temporary tab-separated text file which is processed by the import program specified in the ***Tools →Preferences*** dialog. The output of the import program is then imported to the Rack Positions table, replacing the existing positioning information. See *Appendix A Data import and export, on page 193* for more details.

Function	Description
Simple Position Import	Imports positioning details from an external file. Details of the import settings and file format are described in *Appendix A Data import and export, on page 193*.
Export Positions	Exports the data in the positioning table to a tab-separated text file. See *Appendix A Data import and export, on page 193* for details of the exported file format.
Print Rack Positions	Prints a copy of the rack positions diagram and table.
Print Method	Prints a copy of the current template (excluding rack positions).

Automatic positioning

Samples and reagents are placed in the rack by region, defined automatically from the content of the method template. Samples within a region are kept together as far as possible. The settings for automatic positioning control how the positioning is applied.



Setting	Description
Region	Lists the sample and reagent regions.
Color	Sets the display color for the region.
Orientation	Determines whether samples are arranged by ***column*** (vertically in the rack and plate diagram) or ***row*** (horizontally in the diagram).
Anchor	Determines the position for the first sample in the region.
Rack	Controls whether the samples and reagents will be placed in the reagent rack or the sample microplate. If ***Auto*** is chosen, placement is decided on the basis of number and volume of solutions in the region.

Setting	Description
Vial size	Determine the vial size for reagents. If ***Auto*** is chosen, placement is determined on the basis of the volume of solution. The vial size is ignored for rack type ***Sample***.
Pooling	Allows solutions with the same name to be combined into one position or to split combined solutions into separate positions for each cycle. Choose ***Yes*** to pool solutions if suitable vial positions are available, or ***No*** if you always want separate positions for each cycle. Choose ***Auto*** to set the pooling according to the internal default settings for the type of region.
Sort by	Solutions within a region may be sorted by one or two parameters.

Use the ***Move up*** and ***Move down*** buttons to change the order in which regions are listed. Regions are placed in the specified rack or plate in the order listed, so that changing the order of the table can change the automatic positioning of samples and reagents.

6 Introduction to evaluation

About this chapter

This chapter provides an introduction to Biacore S200 Evaluation Software, including common display functions and automation of evaluation processes using evaluation methods.

In this chapter

Section		See page
6.1	Starting evaluation	74
6.2	Evaluation interface	75
6.3	Common functions	79
6.4	Evaluation methods	82

6.1 Starting evaluation

Starting the Evaluation software

Start Biacore S200 Evaluation Software from Windows. The software will start with the ***Data*** button expanded in the navigation panel at the left.

The Evaluation Software can also be started from the Control Software using ***Tools →Biacore S200 Evaluation Software*** in the main menu. When opened this way, the file currently open in the Control Software will be opened in the Evaluation Software. If the run is still in progress in the Control Software, the most recently saved snapshot will be opened for evaluation without disturbing the on-going run. If no file is open in the Control Software, the ***Open*** dialog will be presented automatically.

Opening files

Click ***Open*** in the navigator to open result files (file type ***.blr***) or saved evaluation sessions (file type ***.bme***). If a file is already open in the software, opening a new file will automatically close the first file. You will be prompted to save the file if any changes have been made.

Any result file can be opened in the Evaluation Software and evaluated using generic tools such as sensorgram display, QC plots and result plots (see *Section 6.2 Evaluation interface, on page 75*). Some application-specific evaluation procedures impose special requirements on the data in the result file. These requirements will generally be met if runs are performed using methods based on pre-defined templates. In some cases of results derived from custom method templates, changes to the keyword table or custom report points may be required to meet the requirements.

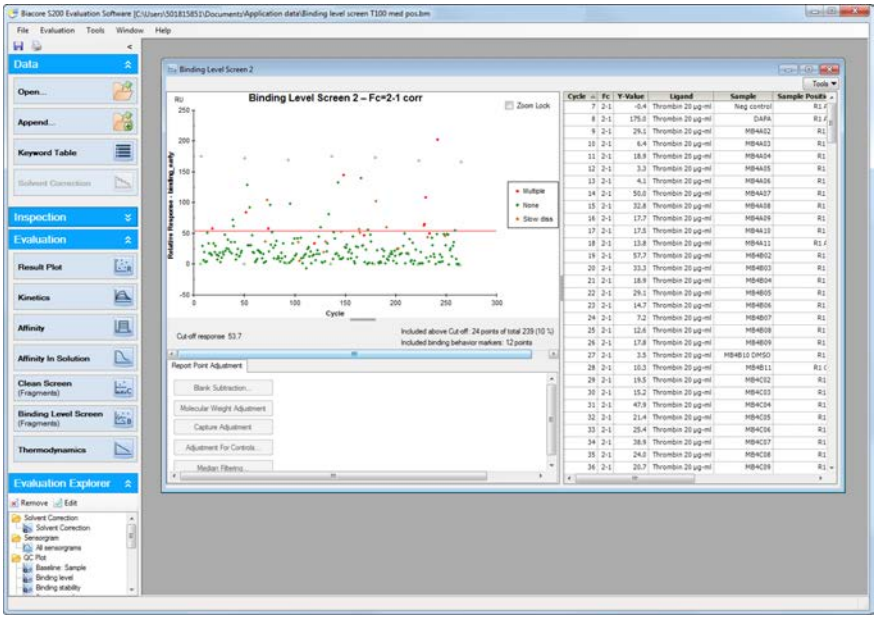
To open multiple result files in the same session, select multiple result files in the ***Open*** dialog or use the ***Append*** button in the navigator. You can only append result files, not evaluation sessions. Appending a file to a session will delete all the user-defined evaluation items in the session.

Note: *The software does not check that multiple files opened in the same session contain compatible data.*

6.2 Evaluation interface

Screen panels

The Evaluation Software screen is divided into a navigator panel on the left and the main workspace on the right. Buttons in the navigator provide access to the most important functions in the software, arranged in groups for ***Data***, ***Inspection***, ***Evaluation*** and ***Evaluation Explorer***. The menu at the top of the window provides additional functions as well as alternative access to functions in the navigation panel.



Note: *Clicking a button in the navigator will create a new evaluation item. Use the Evaluation Explorer to open existing items.*

Evaluation items are opened in a new window during creation and processing. Finished items are placed in the main workspace and listed in the Evaluation Explorer. Closing an item in the main workspace removes the item window but does not delete the item from the evaluation session.

Note: *Finished items in the main workspace are shown by default maximized to the workspace area. Use the standard Windows buttons at the top right of the item window to change window display.*

The remainder of this section gives a brief overview of the main components in the Evaluation Software interface. Functions are described in more detail in subsequent chapters.

Data functions

The ***Data*** group covers functions that determine the data available for evaluation in the session.

Button	Function	Details
Open	Opens a result or saved evaluation file.	See *Section 6.1 Starting evaluation, on page 74*.
Append	Appends a result file to an existing evaluation session. All user-defined evaluation items will be deleted and pre-defined items will be recreated.	
Keyword table	Opens the keyword table. If any changes are made to the keyword table, all user-defined evaluation items will be deleted when the changes are applied.	See *Section 7.1 Keywords, on page 85*.
Solvent correction	Creates and applies solvent correction. An evaluation session may only have one solvent correction item: once correction has been applied, the function is no longer available. This function is not available if the result files do not contain solvent correction cycles.	See *Section 7.3 Solvent correction, on page 92*.

Data content in the evaluation session is described in more detail in *Chapter 7 Data content, on page 84*.

Inspection functions

The ***Inspection*** group covers functions primarily intended for examining the data. Some functions may also be useful in preparing data for presentation purposes.

Button	Function	Details
Sensorgram	Creates a new sensorgram item.	See *Section 8.2 Sensorgram items, on page 100*.
QC Plot	Creates a new QC plot item.	See *Section 8.3 QC plots, on page 107*.

Button	Function	Details
Report Point Table	Creates a report point table item. An evaluation session may only have one report point table item. One is created by default, so this function is not available unless the report point table is explicitly deleted.	See *Section 8.4 Report point table, on page 112*.

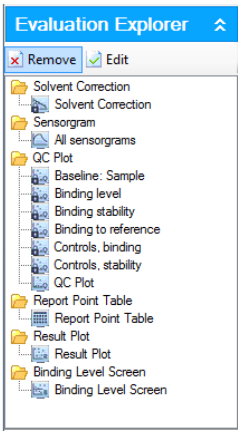
Evaluation functions

The ***Evaluation*** group covers functions that process the data and deliver a result.

Button	Function	Details
Result Plot	Creates a new result plot item.	See *Chapter 9 Result plots, on page 115*.
Kinetics	Creates a new item for kinetics evaluation.	See *Chapter 11 Evaluating kinetics and affinity, on page 142*.
Affinity	Creates a new item for steady state affinity evaluation.	
Affinity In Solution	Creates a new item for evaluation of affinity in solution.	See *Chapter 13 Affinity in solution, on page 179*.
Clean Screen	Creates a new ***Clean Screen*** item.	See *Section 10.1 Clean screen, on page 134*.
Binding Level Screen	Creates a new ***Binding Level Screen*** item.	See *Section 10.2 Binding level screen, on page 138*.
Thermodynamics	Creates a new ***Thermodynamics*** item.	See *Chapter 14 Thermodynamic analysis, on page 185*.

Evaluation explorer

The ***Evaluation Explorer*** lists all evaluation items in the current session. Items are organized in folders according to type.



- Double-click on a folder to expand or collapse the folder.
- Click on an item to display it in the work area.
- Right-click in the explorer area for options for adding new items.
- Right-click on an item for additional options relating to the item.
- Select an item and use the ***Remove*** or ***Edit*** buttons at the top of the Evaluation Explorer to delete or edit the item respectively.

6.3 Common functions

Zooming the display

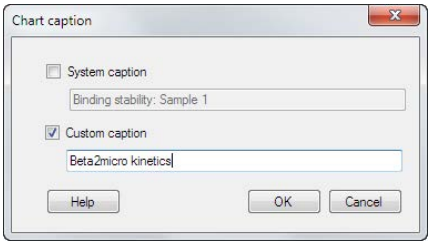
To zoom a display window, drag with the mouse over the area you want to enlarge. To restore the previous zoom level, double-click anywhere in the display window except on the axes or legend, or select ***Unzoom*** from the right-click menu.

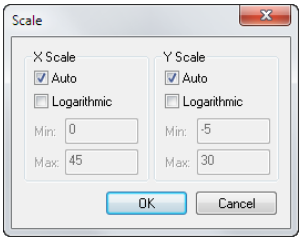
Displays are normally rescaled automatically whenever you change the displayed data. To keep the current zoom setting when data is changed, check ***Zoom lock*** in the display window.

You can also set the display scale with the ***Scale*** option from the right-click menu. The display is not rescaled automatically if the scale has been set to specified values.

Right-click menus

Right-click in display windows for options relating to the display. The available options vary according to the type of window, and also depending on whether you right-click on a point, a curve or elsewhere in the window. Common functions are listed in the following table.

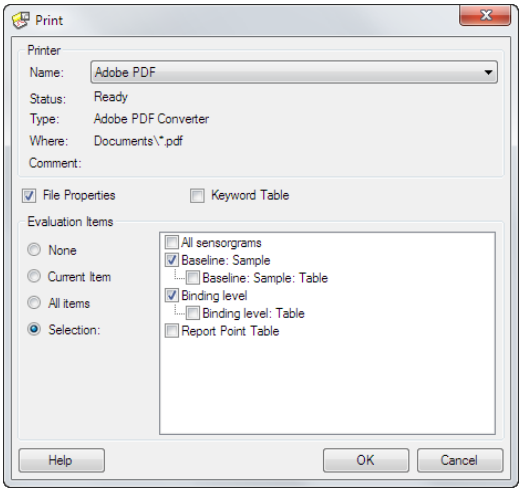
Option	Description
Labels	Displays a label on each point in a plot window, showing cycle number, flow cell and sample name. (Labels may overlap and be difficult to read if the points in a plot are closely spaced.)
Caption	Sets a caption for the item window. The displayed caption can have system defined and user-defined components. 
Show sensor-gram	Displays the sensorgram relating to a point in the plot. This option is only available when you right-click on a point: the sensorgram is displayed in a separate window that must be closed before you can continue with the evaluation.

Option	Description
Exclude/Include	Excludes data from the evaluation session or item. The data that can be excluded may be ***cycle***, ***curve*** or ***point*** depending on the type of window. Excluding data in sensorgram or QC plot windows affects all other sensorgram and QC plot windows correspondingly. Other windows are not affected until they are edited and updated. Excluding data in windows other than sensorgram and QC plot affects only the item where the exclusion is performed. Multiple cycles can be excluded in items by selecting in the table instead of the plot. Excluded cycles are shown with a broken line in sensorgrams. Excluded points are not displayed in plots but are listed in red strike-through text in the plot table (see *Plot table, on page 108*). Excluded cycles may be re-included using the right-click menu on the sensorgram or in the plot table.
Scale	Sets the scale for the display. This function can also be accessed by double-clicking on either the x- or y-axis in the display.  ***Auto*** setting scales the display according to the data content. Uncheck ***Auto*** and enter minimum and maximum values to specify a scale.
Copy graph	Copies the current display as it appears on the screen to the Windows clipboard as a graphic object, for pasting into third-party software.
Copy table	Copies the table data in the current display to the Windows clipboard as tab-separated text.
Export curves	Exports the coordinates for each point on the curves in the current display to a tab-separated text file, for import to third-party software. Complete data is exported regardless of the scale setting of the screen display.
Unzoom	Restores the previous zoom setting.

Option	Description
Gridlines	Shows or hides major and minor gridlines in the display window.
Legend	Shows or hides a legend for the display window. Choose the legend placing from the dialog. In sensorgram and plot windows, the legend corresponds to the ***Color by*** setting for the display.

Printing results

Choose ***File*** →***Print*** to print a hard-copy of the results. Select the printer to use and choose the items you wish to print.



6.4 Evaluation methods

Introduction

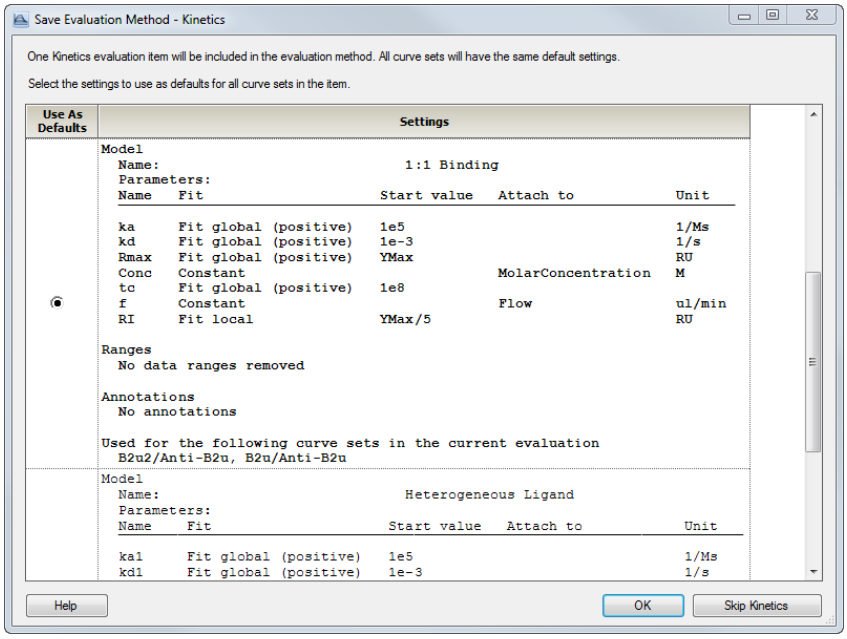
Evaluation methods allow you to save the definitions of the evaluation items in an evaluation session, and apply them automatically to the other result files. Use this feature to apply standardized evaluation procedures to different result files, or to restore an evaluation session after operations that delete user-defined items such as changes made in the keyword table (see *Editing the keyword table, on page 85*).

Create an evaluation method

To create an evaluation method, choose ***File*** →***Save Evaluation Method As***. The method is saved with file type ***.evalmethod***.

All evaluation items in the current session except thermodynamics are included in the evaluation method, with the following limitations:

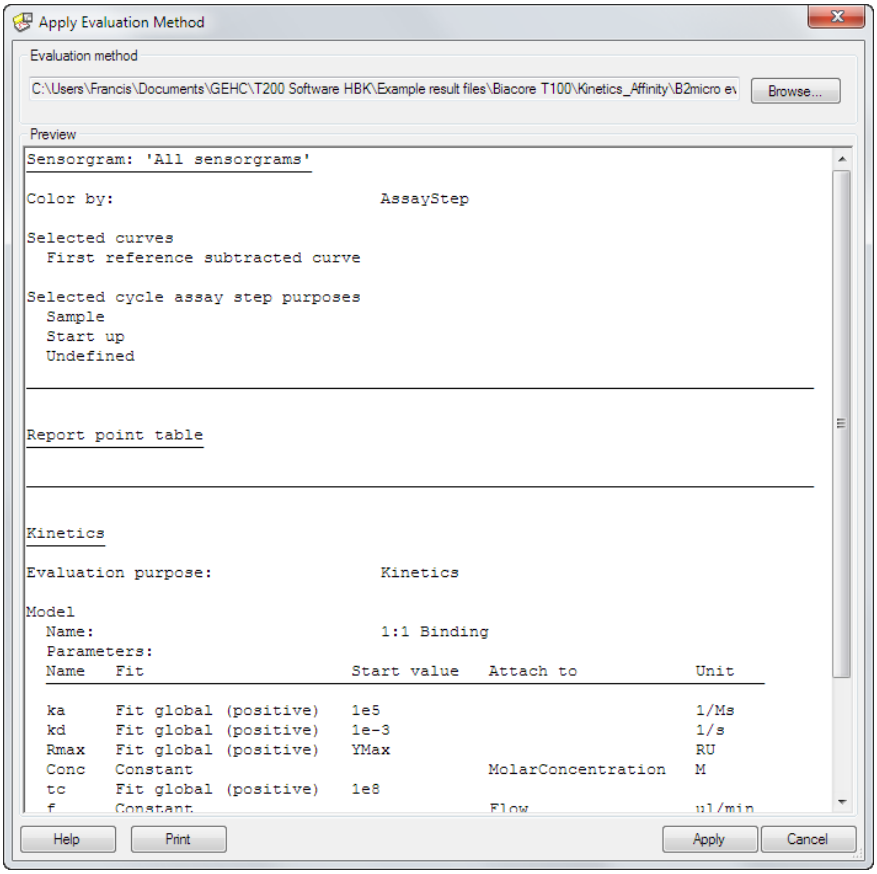
- Custom report points and changes to the keyword table are not included.
- Median filtering settings in result plots are not included.
- Thermodynamics items are not included.
- Status settings for sample series in kinetics/affinity items are not included.
- Kinetics/affinity items that use data sets with multiple R_{max} are not included.
- Only one item each for kinetics and affinity may be included, with only one fitting. If there are more items or fittings in the session, you will be asked which one you want to include in the method.



Apply an evaluation method

To apply an evaluation method to the contents of an evaluation session, choose ***File →Apply Evaluation Method*** and choose the method. Any items already defined in the session will be deleted.

A preview of the method is shown so that you can check the method contents.



Click ***Apply*** to apply the method. Items in the method will be applied as far as possible. Items that cannot be created will be reported in a dialog.

For kinetic and affinity items, applying an evaluation method will create the items but will not perform the fitting automatically. The same model settings will be applied to all sample series in the result file. Edit the item to adjust the settings if necessary and complete the fitting.

7 Data content

About this chapter

This chapter describes functions that determine the data available in the evaluation session, accessed from the ***Data*** group in the navigator panel.

In this chapter

Section		See page
7.1	Keywords	85
7.2	Custom report points	89
7.3	Solvent correction	92

7.1 Keywords

Introduction

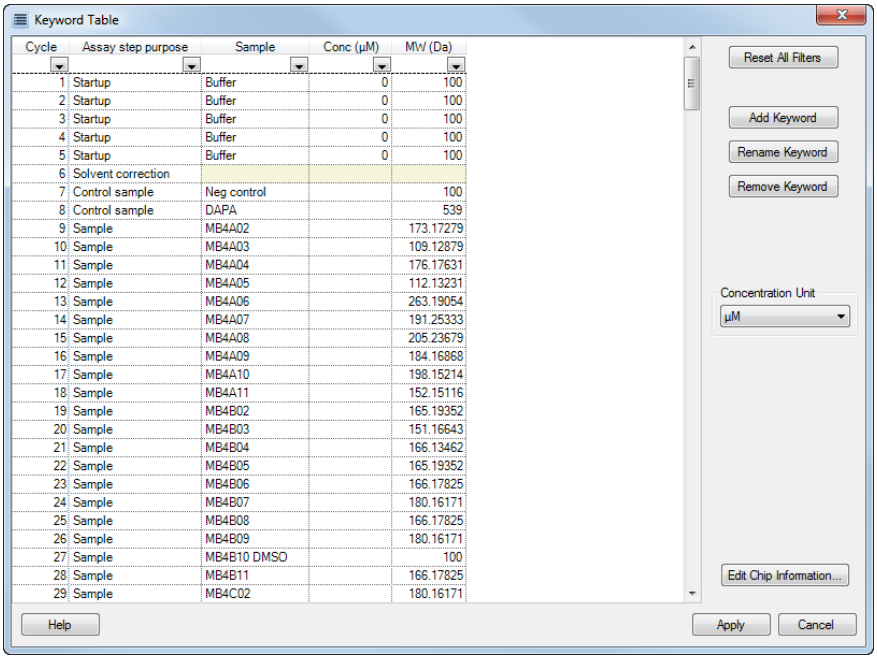
In addition to the actual measurement data from the run, the result file includes keywords that are assigned to cycles when the run is performed, and are then used for identification and evaluation purposes. Keywords can also be created in the evaluation software if necessary. Keywords include:

- names of the molecules immobilized on the sensor surfaces, stored in the ***Chip Properties*** when the sensor chip is prepared (see *Section 4.4 Immobilization results, on page 34*).
- automatically generated identifiers such as cycle number and assay step purpose,
- method variables and evaluation variables (see *Variables, on page 54*).

Keywords are listed and managed in the ***Keyword Table***. You can edit the contents of most keywords.

Accessing the keyword table

Click the ***Keywords*** button in the navigator to access the keyword table.



Editing the keyword table

To simplify management of the keyword table, you can sort and filter the table display:

- Click on a column header to sort the table by the contents of that column.

- Click in the filter row (directly below the column header) and select a value to display only rows with that value for the chosen column. Click ***Reset All Filters*** to restore all filters to the ***[All]*** setting.

To change a keyword value, simply enter the new value in the appropriate cell. Values for some system-generated keywords (such as ***Assay Step Purpose***) are chosen from a predefined list of values: the list is displayed when you click in such a cell. The cycle number cannot be changed. Bear in mind that keywords are listed by cycle. Make sure that you change the value in all cycles where required. You can use **Ctrl-C** (***Copy***) and ***Ctrl-V*** (***Paste***) for values entered from the keyboard.

To change the units for concentration keywords, choose a new unit from the ***Concentration Unit*** list. This changes the unit but not the numerical value of the keyword. For example, a concentration entered as 10 may be for example 10 µM, 10 mM or 10 ng/ml according to the ***Concentration Unit*** setting. If the evaluation session includes data from multiple files, a table of concentration units for the different files is displayed. Make sure that the unit is correct for all files if data are to be evaluated together.

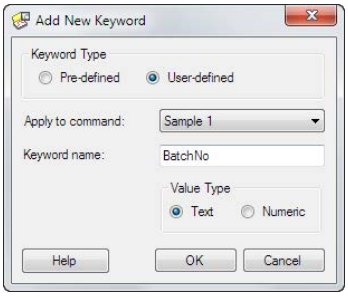
Note: *The concentration unit affects only predefined concentration keywords. User-defined keywords will not be affected when you change the concentration unit even if they are used to hold concentration values.*

When you save changes to the keyword table, all user-defined items in the evaluation session except custom report points will be deleted.

Tip: *Save the current session as an evaluation method if you want to restore user-defined evaluation items after editing the keyword table.*

Adding, renaming and deleting keywords

Click ***Add Keyword*** to create a new keyword in the table. You can choose between predefined keywords and user-defined keywords. If the added keyword is applicable to more than one injection (for example ***Capture*** and ***Sample*** injections), specify the injection to which it should be applied.



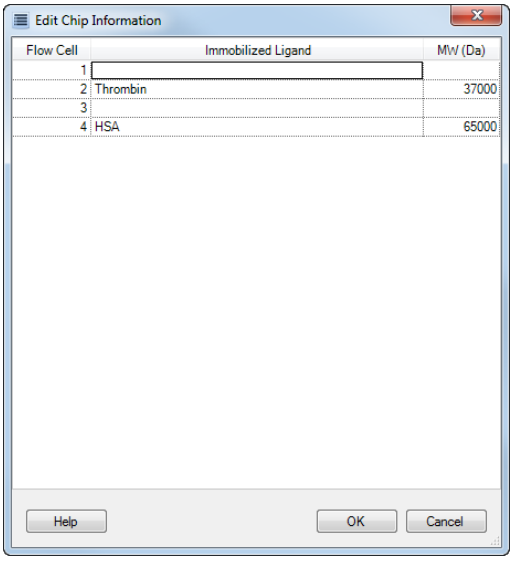
Enter the required keyword values in the empty column that is created for the new keyword.

To rename or delete a keyword, click the appropriate button, then select the keyword in the dialog. You cannot remove system-generated keywords such as file number or cycle number, or keywords derived from method variables such as sample name.

Ligand details

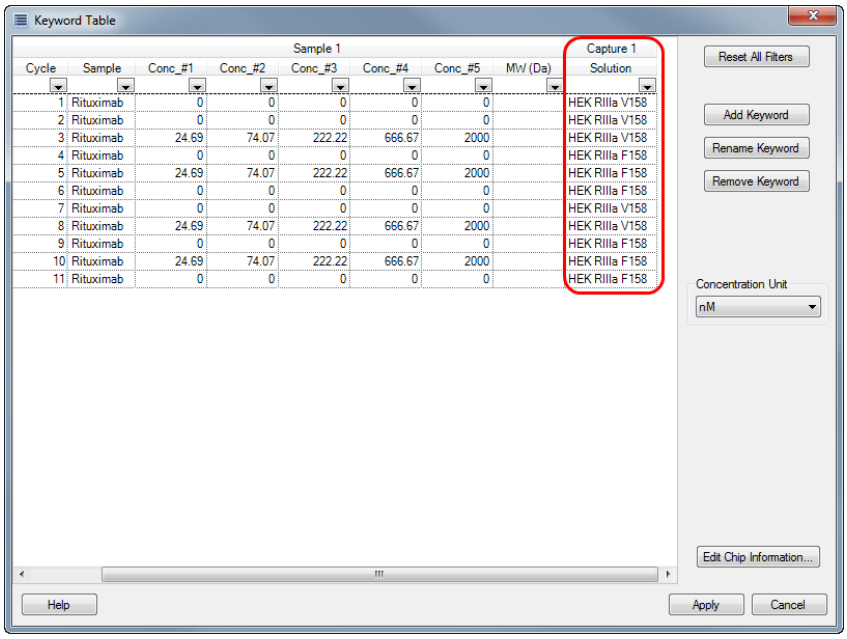
Follow the instructions below to edit the ligand details.

- For ligand directly immobilized on the sensor surface, click ***Edit Chip Information***.



Note: *In capture assays, this dialog shows the details of the capturing molecule, not the ligand.*

- For captured ligand, the name appears as the name of the capture solution in the keyword table and can be edited directly.

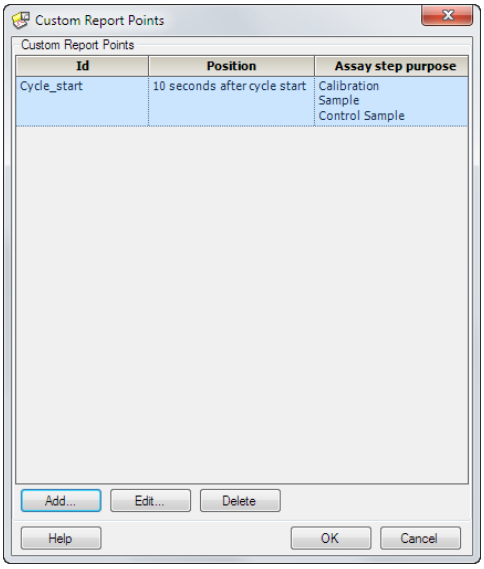


7.2 Custom report points

Introduction

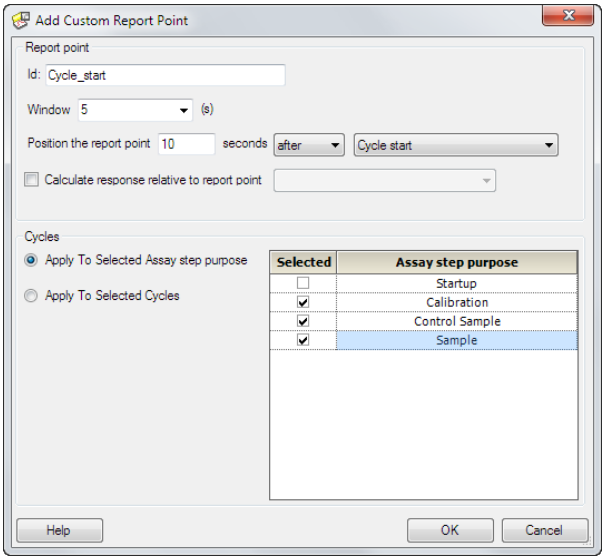
Report points are automatically created for all automated runs, placed at strategic positions in the sensorgrams. These report points, and any other report points that have been created in the Control Software (see *Report points, on page 57*), are not listed in the ***Custom Report Points*** dialog and cannot be edited or deleted in the Evaluation Software.

To add, edit or delete custom report points, choose ***Tools*** →***Custom Report Points***.



Adding report points

Click ***Add*** to add a new report point.



Note: *If you append a result file to the evaluation session after creating custom report points, the custom report points are retained but they are not applied to the appended file. To apply a custom report point to all files, either append all files before creating the report point or edit the report point definition and re-apply without changing the settings.*

Enter the settings as follows.

Setting	Description
Id	Name of the report point (maximum 30 characters). The name must be unique within the evaluation session.
Window	Report point window in seconds. This may be any value between 1 and 35. Preset values of 5, 10 and 15 seconds are provided for convenience.
Position	Defines where the report point is placed in relation to a selected event. You cannot define a report point with position and window settings that would extend outside the range of the sensorgram. ***Note:*** *Do not position report points far away from events so that they lose their relevance to the event, or so close to an event so that the report point window overlaps the event itself.*

Setting	Description
Calculate response	Defines the reference report point for calculation of relative response. If this setting is not checked, the response will be calculated relative to the closest preceding baseline report point. ***Note:*** *You cannot assign the* ***baseline*** *property to a custom report point.*
Cycles	You can apply custom report points either to cycles with a selected assay step purposes or to cycles selected by cycle number. Choose the appropriate option and check the assay step purpose(s) or cycles to which the report point should apply.

Editing and deleting report points

Select a report point in the list in the ***Custom Report Points*** dialog and click ***Edit*** to edit the report point definition or ***Delete*** to delete the report point. You will be warned if deleting a report point has any impact on remaining report points.

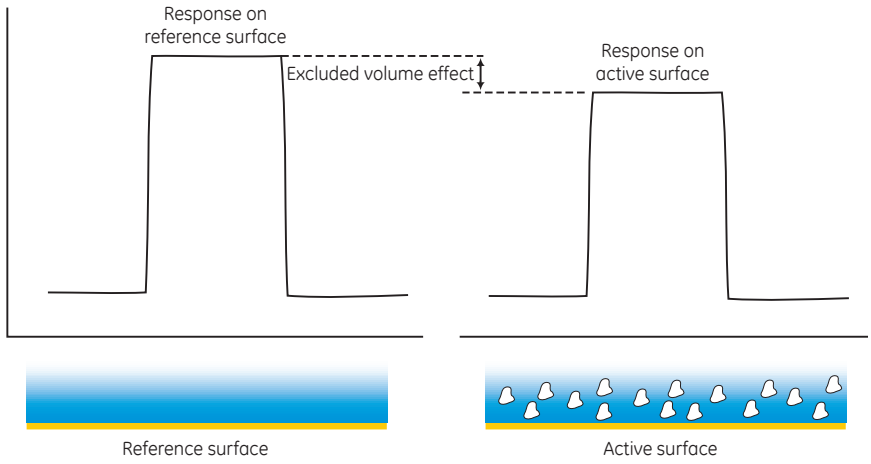
Editing or deleting custom report points will delete all user-defined evaluation items in the session. Other custom report point and changes to the keyword table are not affected.

7.3 Solvent correction

Background

Solvent correction adjusts reference-subtracted responses for small artefacts that can be introduced by variations in the bulk refractive index between samples. The correction is only relevant when variations in the bulk refractive index are of the same order of magnitude as the response: this situation arises commonly in work with small organic analytes that give intrinsically low response values and that often require organic solvents such as dimethyl sulfoxide (DMSO) to maintain solubility.

The need for solvent correction arises when the amount of ligand on the active surface is high compared with the reference, and the bulk refractive index contribution of the solvent is high compared with the expected analyte response. Bulk solution is excluded from the volume occupied by ligand on the active surface, so that subtraction of the reference response does not exactly correct for the solvent contribution. This is schematically illustrated below.



As long as the refractive index of the samples is constant, this excluded volume effect introduces a constant error in reference subtraction which may be ignored for practical purposes. However, if the refractive index of the samples varies, the magnitude of the excluded volume effect will also vary.

Addition of 1% DMSO to buffer gives a bulk response of about 1200 RU, so that small variations in the DMSO content lead to significant variations in the bulk response between samples. Such variations are unavoidable in the preparation of diverse samples such as drug candidates for screening applications. The solvent correction procedure corrects for the variations arising from the excluded volume effect in these cases.

A more detailed description of solvent correction background and procedures may be obtained from Cytiva.

When solvent correction should be used

Solvent correction is only relevant when:

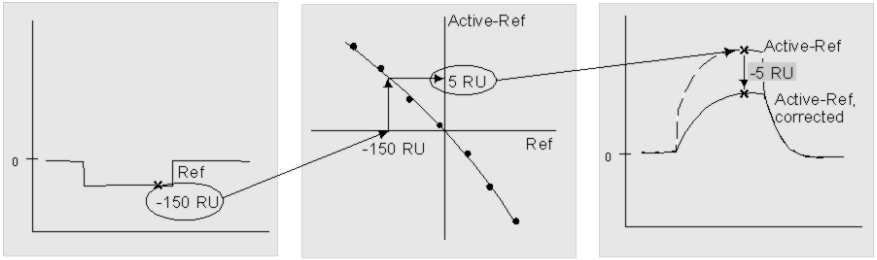
- the expected analyte responses are low,
- the ligand is a macromolecule immobilized at a high density compared to the reference surface (typically 5000 RU or more – lower ligand densities lead to excluded volume effects that are too small to merit correction),
- the bulk response is subject to variations between samples of at least the same order of magnitude as the expected binding response.

Solvent correction should not be applied in situations that do not meet all three of these criteria. Attempts to use solvent correction in other circumstances may introduce errors that are larger than the solvent effects that the procedure is intended to correct.

How solvent correction works

Solvent correction is determined by injecting a series of blank samples containing a range of solvent concentrations over the active and reference surfaces, and plotting the reference-subtracted relative response on the active surface against the relative response on the reference surface. Each sample measurement is then corrected by a factor obtained by measuring the relative response on the reference surface and reading the corresponding difference between active and reference surfaces from the correction curve.

Example



- The sensorgram from the reference flow cell shows a bulk displacement (-150 RU in the illustration) during sample injection because the sample and running buffer are not exactly matched.
- From the solvent correction curve, a displacement of –150 RU in the reference sensorgram corresponds to a solvent error of +5 RU in the reference-subtracted sensorgram.
- The reference-subtracted sensorgram is corrected by subtracting the solvent error. This procedure is applied to every sensorgram point during sample injection.

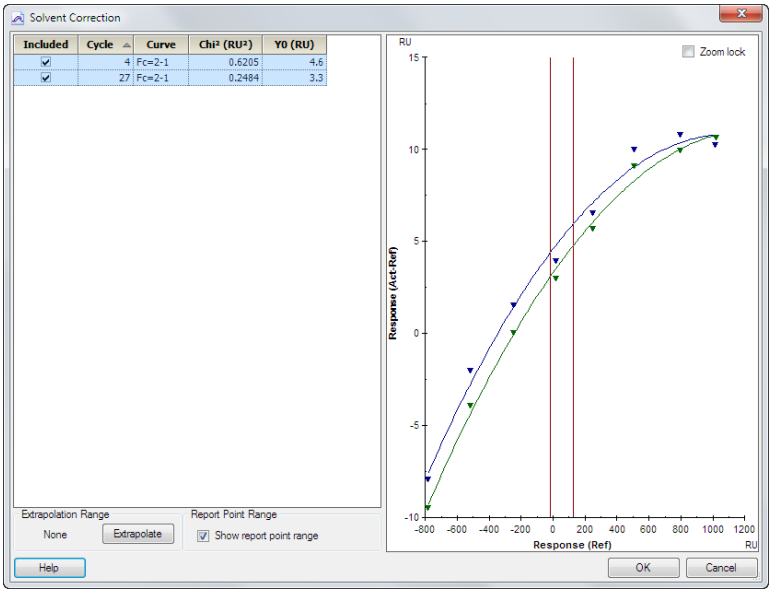
Solvent correction is applied only to response levels during sample injection, since the correction adjusts for differences in the bulk refractive index of the samples compared with running buffer.

Solvent correction procedure

Solvent correction should be applied where appropriate before any other evaluation. Once the correction has been applied, evaluations can be performed on corrected or uncorrected data as required.

Follow the steps below to apply solvent correction.

Step	Action
1	Click the ***Solvent correction*** button in the navigator. *Result:* The ***Solvent Correction*** window opens.



The left-hand panel lists the solvent correction curves in the run, and the selected curves are shown in the right-hand panel. A separate curve is created for each solvent correction cycle and each reference surface. Statistical fitting parameters (chi-square and ***Y0***, the reference subtracted response at zero reference response) are shown for each correction curve. The solvent correction curve is fitted to the experimental points using a second-degree equation.

The range of sample report point values that are candidates for solvent correction is indicated by vertical lines in the window.

2 If a curve does not fit the experimental points acceptably:

Step	Action
	• To exclude a point, right-click on the point and choose ***Exclude point***. The curve will be refitted. • To exclude a curve, either remove the ***Included*** checkmark from the list of curves or right click on the curve and choose ***Exclude curve***. • To examine the sensorgrams for a solvent correction cycle, right click on a curve and choose ***Show sensorgrams***.
3	Click ***OK*** to apply the solvent correction. Correction will be applied to the sample and carry-over injection phase(s) of all sensorgrams. Any data points that lie outside the correction range will be discarded and the corresponding sensorgram will contain gaps corresponding to the invalid data. Solvent correction is only applied to sample measurements made at the same temperature as the correction curve. Once solvent correction has been applied, the item cannot be edited or deleted, and excluding or including solvent correction cycles will have no effect on the correction.
4	Examine the solvent correction curves for curve quality and correction ranges as described below, to determine whether to use corrected or uncorrected data in subsequent evaluation.

Quality assessment

Judge whether to use solvent corrected data or not using the criteria listed in the following table.

Property	Recommendation
Curve quality	The solvent correction curves should be a reasonably close fit to the experimental points. As a rule of thumb, chi-square values should be below 2 RU. Exclude any isolated outlying points from the curves. Beware of applying solvent correction if the correction curve does not fit the experimental points closely. Scatter in the correction points can distort the measured responses unnecessarily. Careful preparation of solvent correction solutions is essential for reducing scatter. ***Note:*** *In judging the quality of the solvent correction data, take note of the y-axis scale in the display. The curves are automatically scaled to fit the window.* The shape and slope of the solvent correction curve (even the direction of slope) may vary between measurements on different occasions. This is normal and the curve shape should not be taken as an indicator of curve quality.
Report point range	If either of the vertical lines indicating the report point range lies outside the curve range, examine a plot of reference response values for the samples to identify which cycles will be affected. Solvent correction cannot be applied to points outside the curve range. Correction curves may be extrapolated if necessary using the ***Extrapolate*** button. However, the shape of the curves is not fully predictable, and extrapolation by more than about 10% of the range of the reference values is not recommended. ***Note:*** *The vertical lines show the range of report point values in the data that will be corrected. Remember however that solvent correction will be applied to all data points in the sensorgram during sample injection.*
Correction range	The y-axis range of the curves between the report point range lines gives an indication of the magnitude of solvent correction for report points. Compare this range with the range of measured response values to judge the effect of solvent correction on the data.

8 Data inspection

About this chapter

This chapter describes tools for data inspection, accessed from the ***Inspection*** group in the navigator.

In this chapter

Section		See page
8.1	Predefined evaluation items	98
8.2	Sensorgram items	100
8.3	QC plots	107
8.4	Report point table	112

8.1 Predefined evaluation items

Introduction

When a result file is opened, a number of evaluation items are created automatically if the results contain the appropriate cycles and report points.

Sensorgram

An overlay plot of all sensorgrams is created automatically. The sensorgrams are colored by assay step. No adjustments are applied.

QC plots

QC plots are created automatically if the appropriate report point is present in the results. The settings for predefined plots are locked and cannot be edited. Common predefined plots are listed below. Separate plots will be created if there are multiple injections with similar report points (for example baseline for capture and sample injections).

Plot item	Description
Baseline →Sample	Absolute response for report point ***baseline*** against cycle number.
Baseline →Capture	Absolute response for report point ***capture_baseline*** against cycle number.
Baseline →General	Absolute response for report point ***general_baseline*** against cycle number.
Binding to reference	Relative response for report point ***stability*** against cycle number for the reference flow cell.
Capture	Relative response for report point ***capture_level*** against cycle number for the capture injection.
Carry-over	Relative response for the report point ***co_binding*** against cycle number for the carry-over injection.
Controls, binding	Relative response for the report point ***binding*** against cycle number for control samples.
Controls, stability	Relative response for the report point ***stability*** against cycle number for control samples.
Binding levels	Relative response for the report point ***binding*** against cycle number for samples (only for reference-subtracted curves).
Binding stability	Relative response for the report point ***stability*** against cycle number for samples.

Plot item	Description
Binding to reference, enhancement	Relative response for report point ***enhance_level*** against cycle number for enhancement injections on the reference surface.
Enhancement	Relative response for the report point ***enhance_level*** against cycle number for enhancement injections on the active surface.

Report point table

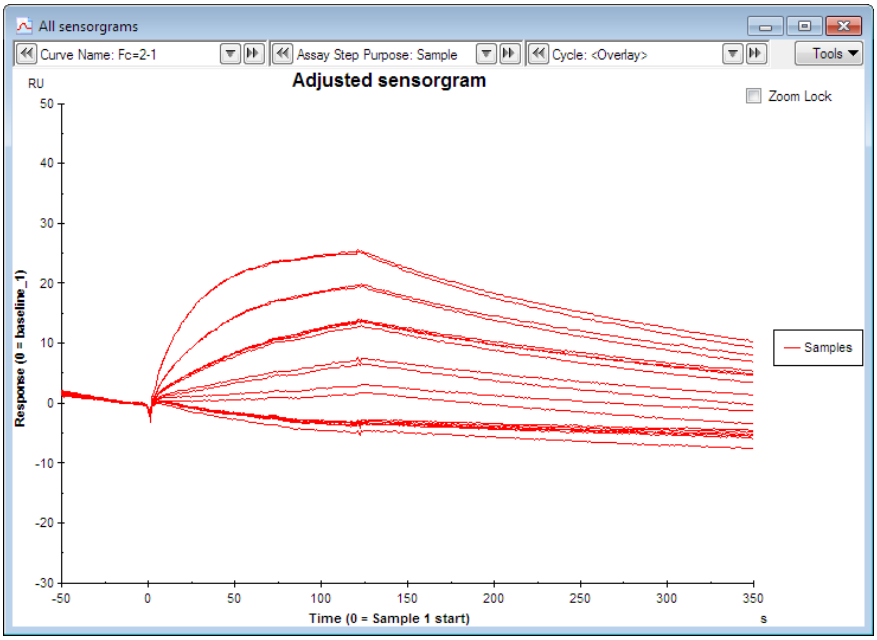
A report point table item is automatically created when the result file is opened. An evaluation session may only contain one report point table.

8.2 Sensorgram items

Introduction

Sensorgram items display sensorgrams from one or more cycles in an overlay plot.

A sensorgram item containing all sensorgrams in the result file is created automatically in the Evaluation Explorer when the file is opened. You can change the display settings in this item, or create additional sensorgram items if required. Click the ***Inspection*** →***Sensorgram*** button in the navigator panel or choose ***Evaluation*** →***Add sensorgram*** to add a new sensorgram item. A new sensorgram item displays all sensorgrams by default.

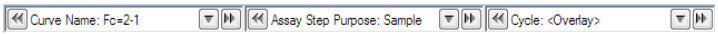


Hold the cursor over a curve to display a tool tip identifying the curve.

The following sections describe display functions specific to sensorgram items. General display functions are described in *Section 6.3 Common functions, on page 79*.

Selecting sensorgrams for display

The selector bar at the top of the window controls which sensorgrams will be displayed.



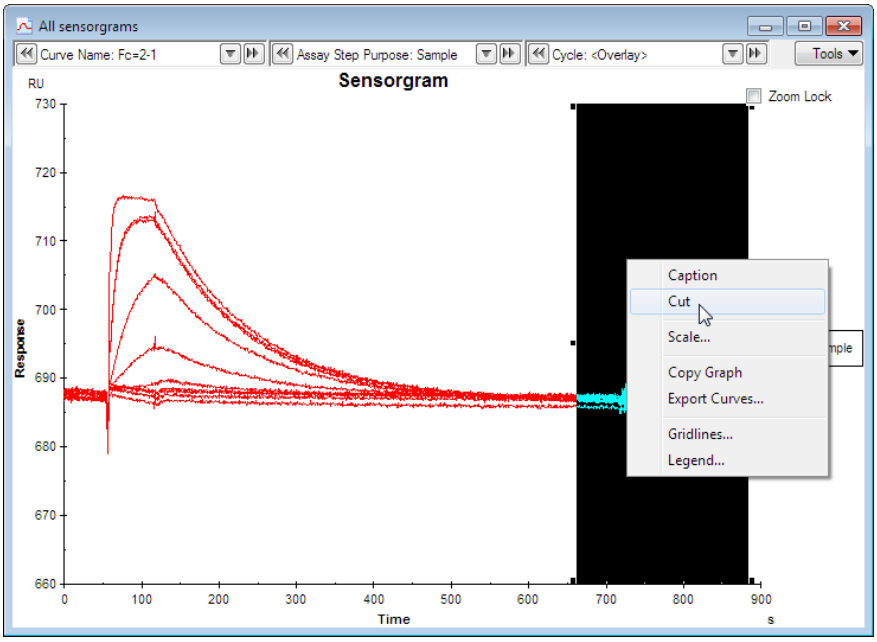
- ***Curve name*** filters the sensorgrams according to the flow cell and type of sensorgram (active, reference, reference subtracted and solvent corrected where applicable).
- ***Assay Step Purpose*** filters the sensorgrams according to the assay step purpose.
- ***Cycle*** filters the sensorgrams according to cycle number (and file number if multiple files are open).

For each filter:

- Click the browse buttons (,) to browse backwards or forwards through the list, one item at a time.
- Click the selector button () to open the list for selecting one or more items. Drag with the mouse or use **shift-click** and **ctrl-click** to select multiple items. To accept a selection, click anywhere outside the list or press **Enter**.

Removing data

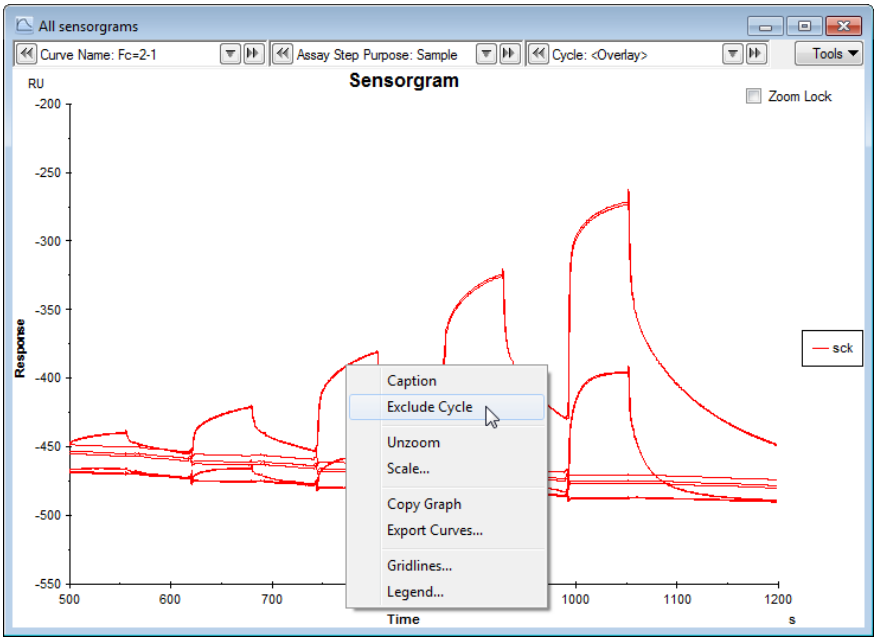
To remove data from the display, mark the section to be removed by dragging with the right mouse button, then choose ***Cut*** from the right-click menu. The data will be removed from the current sensorgram display item only. No other windows or evaluation items will be affected. This function can be useful for removing injections with high bulk contributions (such as regeneration injections) or other visual disturbances from the display.



Choose ***Undo Cut*** from the right-click menu to restore the removed data.

Excluding cycles

To exclude a cycle from the evaluation, right-click on the sensorgram and choose ***Exclude Cycle***. The sensorgram will be displayed with a broken line, and will be excluded from all subsequently created or edited evaluation items. Existing items will however not be affected until they are edited.



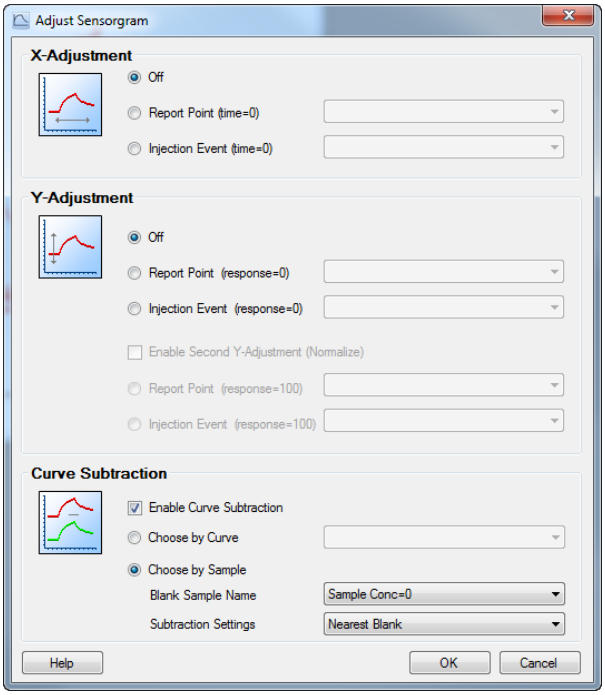
Right-click on an excluded sensorgram and choose ***Include cycle*** to restore the excluded cycle.

Coloring sensorgrams

Choose ***Tools*** →***Color by*** to determine how sensorgrams are colored. Available options are based on the keywords in the evaluation session. Colors are identified in the sensorgram legend.

Adjusting sensorgrams

Choose ***Sensorgram adjustment*** under the ***Tools*** button for options for aligning and adjusting the sensorgram display. For alignment, sensorgrams that do not include the chosen reference point for alignment will not be shown. Sensorgram adjustment only affects the display in the current sensorgram item, and is not related to the adjustment functions in result plots (see *Section 9.4 Data adjustment functions, on page 123*.



X-adjustment

Choose an option under ***X-Adjustment*** to set the zero time point to either a report point or an injection event. If this setting is ***Off***, the zero time point will be at the beginning of the cycle.

Y-adjustment

Choose an option under ***Y-Adjustment*** to set the zero response point to either a report point or an injection event. If this setting is ***Off***, the actual response values will be shown.

Check ***Enable Second Y-Adjustment*** to normalize the response data on a scale of 0 to 100. Each sensorgram will then be normalized separately to zero and 100 at the first and second adjustment points respectively. This can help in comparing the shapes of sensorgrams independently of their response levels, or in adjusting response levels that are dependent on others (e.g. adjusting analyte response for varying capture levels, by adjusting the baseline to 0 and the capture level to 100).

Curve subtraction

Check ***Enable Curve Subtraction*** and choose the subtraction mode.

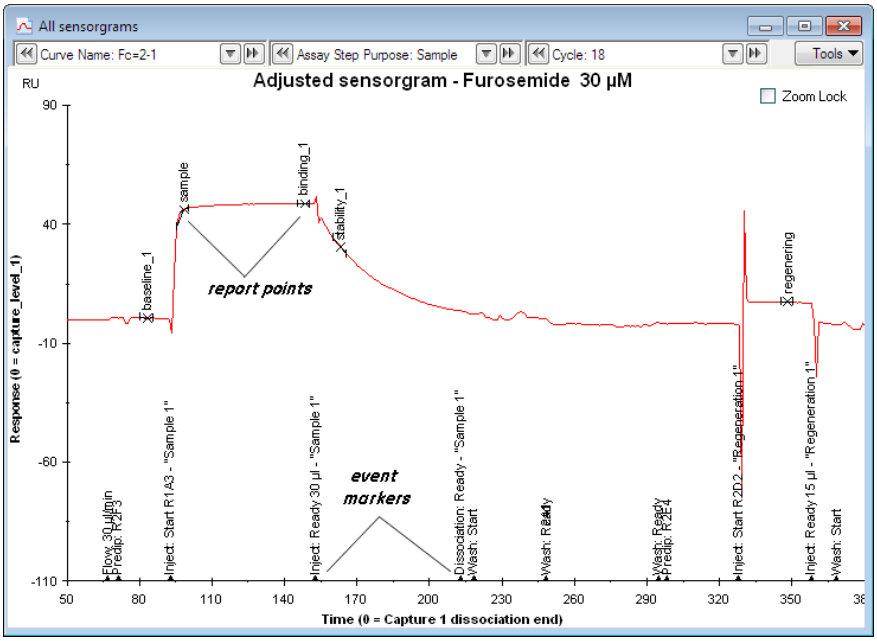
Mode	Description
Choose by curve	Choose one curve that will be subtracted from all others. Subtraction is applied to all curves regardless of flow cell and response type.
Choose by sample	1. Choose the sample to be used as blank. Blank samples are either controls or sample cycles with zero concentration. 2. Choose how the blank cycles should be selected according to the following table of options.

Setting for *Choose by Sample*	Description
Nearest Blank	Nearest blank in the cycle sequence.
Average Nearest Blanks	Average of the nearest preceding and nearest following blanks. If there is no preceding or following blank, the nearest blank is used.
Preceding Blank	Nearest preceding blank in the cycle sequence. If there is no preceding blank, the nearest blank is used.
Following Blank	Nearest following blank in the cycle sequence. If there is no following blank, the nearest blank is used.

Note: *Subtracting a blank sensorgram is not the same as using reference-subtracted data. Reference subtraction gives the difference between active and reference values for each cycle separately, whereas blank subtraction subtracts one cycle from one or more others.*

Markers

Choose to display markers and/or labels for report points and events in the cycle using the ***Report points*** and ***Event markers*** options respectively under the ***Tools*** button. Report points are displayed on the curve and event markers on the x-axis.



8.3 QC plots

Introduction

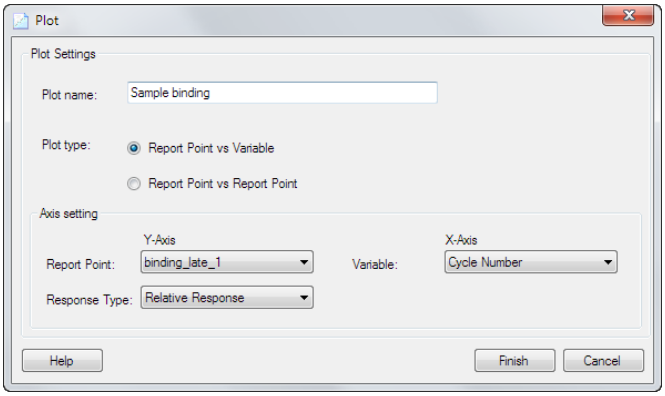
QC plots are intended primarily for presentation and quality control of data. Cycles that are excluded in QC plots are excluded automatically from all other subsequently created or edited evaluation items. Adjustment of response values for analyte molecular weight and ligand capture level (where appropriate) performed in user-defined QC plots affect only the current plot. Additional report point evaluation facilities are provided in result plots (see *Chapter 9 Result plots, on page 115*).

Note: *A number of QC plots are created automatically according to the content of the result file (see Section 8.1 Predefined evaluation items, on page 98). These predefined plots cannot be edited or adjusted.*

Creating QC plots

QC plots display report point values plotted against either variables or other report point values in the same cycle.

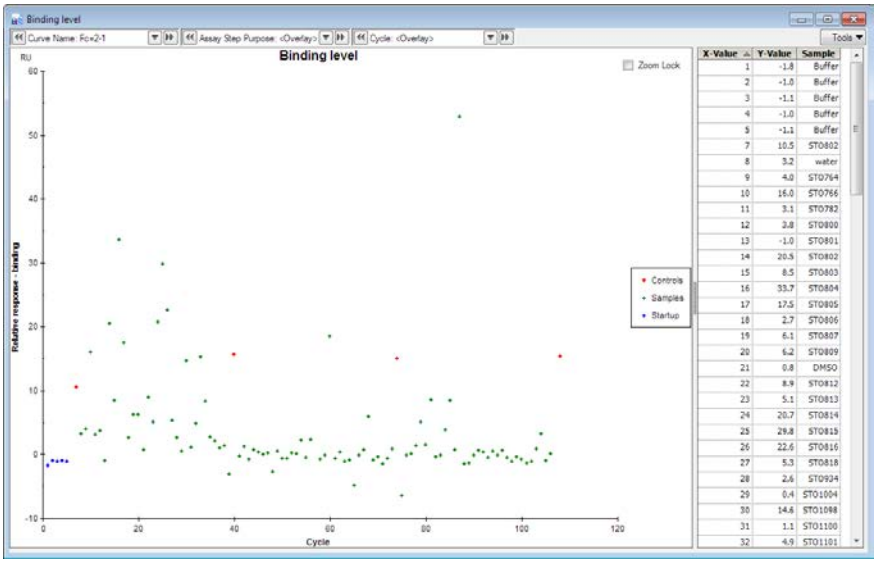
To create a QC plot item, click the ***Inspection*** →***QC Plot*** button in the navigator panel or choose ***Add Plot*** →***QC Plot*** from the ***Evaluation*** menu. Enter a name for the plot, choose the parameters that define the plot and click ***Finish***. Cycles that do not contain the selected report point(s) will not be represented in the plot.



Response type may be response (absolute or relative) or sensorgram slope at the selected report point.

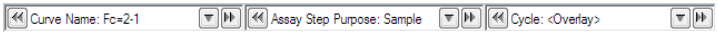
Variables may be numerical (e.g. molecular weight or concentration) or non-numerical (e.g. sample name or assay step purpose). Plots with non-numerical variables on the x-axis are grouped by the selected variable.

The plot will be created with default display settings, with a graphical representation at the left and a table of selected data at the right. Tool tips identify the data points (place the cursor on a point for a couple of seconds to display the tool tip).



Selecting points for display

The selector bar at the top of the window controls which points will be displayed.



Selection operates in the same way as in the sensorgram window (see *Selecting sensorgrams for display, on page 101*). The right-hand filter lists the variable values represented on the x-axis. This option is not available for plots of report point against report point.

See *Section 6.3 Common functions, on page 79* for other general display functions.

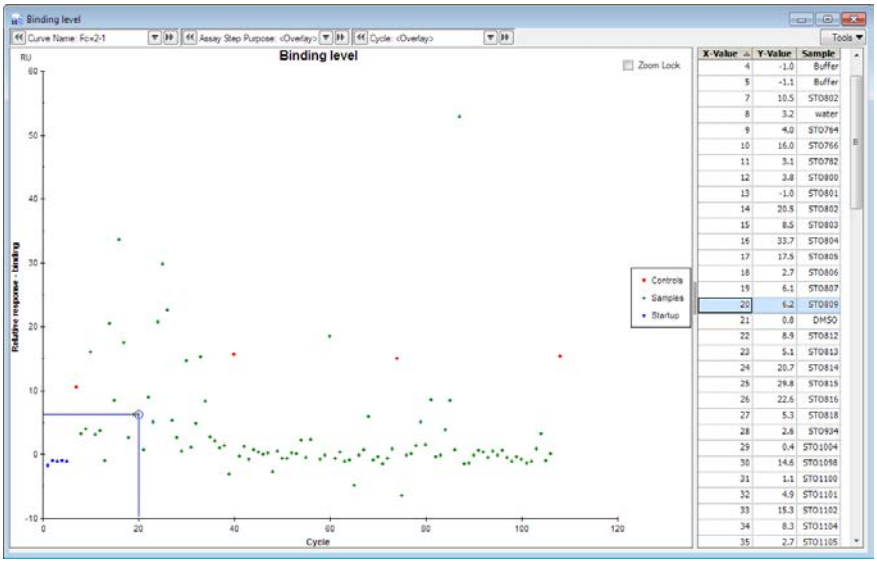
Coloring plot points

Choose ***Tools*** →***Color by*** to determine how plot points are colored. Available options are based on the keywords in the evaluation session. Colors are identified in the plot legend.

Plot table

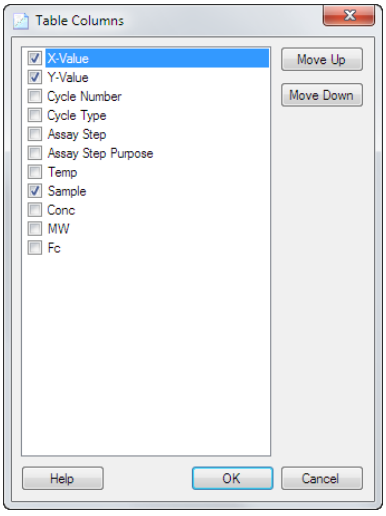
The table to the right of the plot area lists values and sample names for the points in the plot. You can display sensorgrams and exclude or include cycles from the right-click menu in the table area, in the same way as from the right-click menu in the plot. The table also allows you to exclude or include multiple cycles in a single operation. Excluded points are shown struck out in red text.

Select rows in the table to highlight the corresponding points in the plot. If you select a single row, the highlight is augmented with lines drawn to the plot axes.



By default, the table shows x- and y-values sample name and cycle information and is sorted in ascending order of x-values. Click on a column header to sort the table by that column and to change the sort order. Sorting the table does not have any effect on the plot display.

Choose ***Tools*** →***Table columns*** to select columns that will be displayed in the table. You can also change the order in which columns will be displayed using the ***Move up*** and ***Move down*** buttons (the top of the column list represents the left-hand column in the table).



Sorting a plot

QC plots of report point values against variables can be sorted in order of ascending or descending y-axis value, regardless of the variable chosen for the x-axis. Plots of report point against report point cannot be sorted.

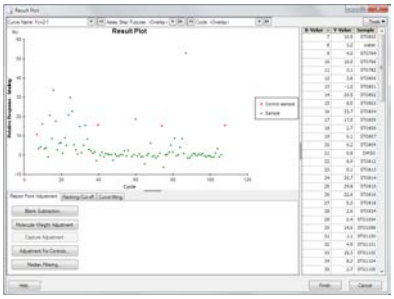
Choose ***Sort*** →***Ascending*** or ***Descending*** under the ***Tools*** button to sort the plot points according to the response values. Choose ***Sort*** →***As defined*** to restore the original display order. Sorting the plot also sorts the rows in the table.

Note: *By default, the table associated with a sorted plot retains a column headed* ***X-Value****. This is the value of the variable originally defined for the plot. Values displayed on the x-axis of a sorted plot are simply sequential numbers.*

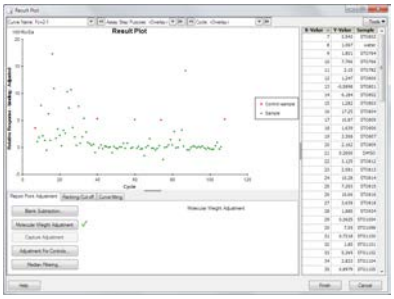
Adjusting for molecular weight

In some situations, quality control of the data may be easier if the results are adjusted for the molecular weight of the analyte, so that the displayed response levels are molar rather than weight-based. To apply this adjustment, choose ***Tools*** →***Molecular weight adjustment***. The adjustment is performed by dividing the response in RU by the molecular weight in Da and multiplying the result by 100 (units RU/100 Da). Points for which the molecular weight value is zero or missing are omitted from the adjusted plot.

Molecular weight adjustment cannot be applied to pre-defined QC plots.



Before molecular weight adjustment



After molecular weight adjustment

Adjustment for molecular weight applies only to the QC plot is which it is selected, and does not affect report points displayed in other plot items. A corresponding adjustment function is provided in result plots (see *Chapter 9 Result plots, on page 115*).

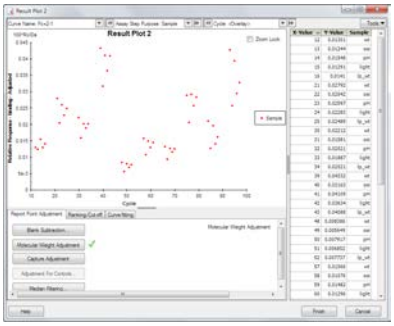
Note: *Editing the definition of a molecular weight-adjusted plot will cancel the adjustment.*

Adjusting for capture levels

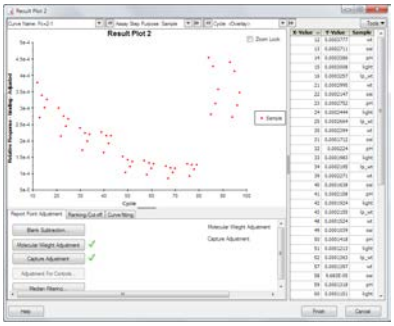
Capture adjustment corrects sample responses for variations in the levels of captured ligand between cycles by dividing the sample response with the response for captured ligand. To apply this adjustment, choose ***Tools*** →***Capture Adjustment***. When capture adjustment has been applied, adjusted response levels will be expressed as sample response divided by capture level. Both axes can be adjusted independently in a plot of report point against report point.

Capture adjustment relies on response values from the report points ***capture_baseline*** and ***baseline*** to determine the capture level. The adjustment cannot be applied if these report points are not present.

Capture adjustment cannot be applied to pre-defined QC plots.



Before capture level adjustment



After capture level adjustment

Note: *Adjustment for capture should only be applied to report points that represent analyte response (either direct or enhanced). The adjustment is not appropriate for report points placed before the sample injection.*

Beware of applying adjustment for capture to report points with significant bulk response contribution. The bulk component will be included in the adjustment and will distort the results.

Editing the definition of a capture-adjusted plot will cancel the adjustment.

8.4 Report point table

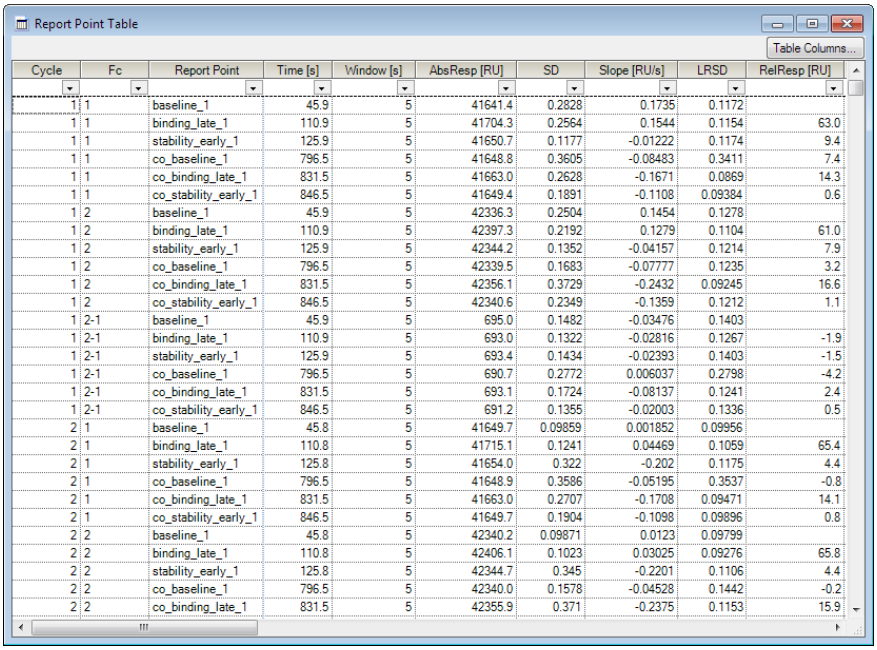
Introduction

The report point table is created automatically when a result file is opened in the Evaluation Software, and lists values for all report points in the run. An evaluation session may only contain one report point table item. The item is updated automatically if you add custom report points or apply solvent correction, but the contents of the report point table cannot be edited directly.

The report point table is not affected by exclusion of cycles in sensorgram or QC plot items.

Values in the report point table cannot be edited, but the contents can be customized by sorting, filtering and selecting columns. Sorting, filtering and column selection are applied to the report table as exported to Excel™ or XML, but not to export in tab-separated text format (see *Appendix A.1 Exporting data, on page 194*).

Report point table display



Cycle	Fc	Report Point	Time [s]	Window [s]	AbsResp [RU]	SD	Slope [RU/s]	LRSD	RelResp [RU]
1	1	baseline_1	45.9	5	41641.4	0.2828	0.1735	0.1172	
1	1	binding_late_1	110.9	5	41704.3	0.2564	0.1544	0.1154	63.0
1	1	stability_early_1	125.9	5	41650.7	0.1177	-0.01222	0.1174	9.4
1	1	co_baseline_1	796.5	5	41648.8	0.3605	-0.08483	0.3411	7.4
1	1	co_binding_late_1	831.5	5	41663.0	0.2628	-0.1671	0.0869	14.3
1	1	co_stability_early_1	846.5	5	41649.4	0.1891	-0.1108	0.09384	0.6
1	2	baseline_1	45.9	5	42336.3	0.2504	0.1454	0.1278	
1	2	binding_late_1	110.9	5	42397.3	0.2192	0.1279	0.1104	61.0
1	2	stability_early_1	125.9	5	42344.2	0.1352	-0.04157	0.1214	7.9
1	2	co_baseline_1	796.5	5	42339.5	0.1683	-0.07777	0.1235	3.2
1	2	co_binding_late_1	831.5	5	42356.1	0.3729	-0.2432	0.09245	16.6
1	2	co_stability_early_1	846.5	5	42340.6	0.2349	-0.1359	0.1212	1.1
1	2-1	baseline_1	45.9	5	695.0	0.1482	-0.03476	0.1403	
1	2-1	binding_late_1	110.9	5	693.0	0.1322	-0.02816	0.1267	-1.9
1	2-1	stability_early_1	125.9	5	693.4	0.1434	-0.02393	0.1403	-1.5
1	2-1	co_baseline_1	796.5	5	690.7	0.2772	0.006037	0.2798	-4.2
1	2-1	co_binding_late_1	831.5	5	693.1	0.1724	-0.08137	0.1241	2.4
1	2-1	co_stability_early_1	846.5	5	691.2	0.1355	-0.02003	0.1336	0.5
2	1	baseline_1	45.8	5	41649.7	0.09859	0.001852	0.09956	
2	1	binding_late_1	110.8	5	41715.1	0.1241	0.04469	0.1059	65.4
2	1	stability_early_1	125.8	5	41654.0	0.322	-0.202	0.1175	4.4
2	1	co_baseline_1	796.5	5	41648.9	0.3586	-0.05195	0.3537	-0.8
2	1	co_binding_late_1	831.5	5	41663.0	0.2707	-0.1708	0.09471	14.1
2	1	co_stability_early_1	846.5	5	41649.7	0.1904	-0.1098	0.09896	0.8
2	2	baseline_1	45.8	5	42340.2	0.09871	0.0123	0.09799	
2	2	binding_late_1	110.8	5	42406.1	0.1023	0.03025	0.09276	65.8
2	2	stability_early_1	125.8	5	42344.7	0.345	-0.2201	0.1106	4.4
2	2	co_baseline_1	796.5	5	42340.0	0.1578	-0.04528	0.1442	-0.2
2	2	co_binding_late_1	831.5	5	42355.9	0.371	-0.2375	0.1153	15.9

The report point table does not list response values adjusted for molecular weight or capture level.

Click ***Table Columns*** to select which columns to display. The available columns are listed in *Report table columns, on page 113*.

Click on a column header to sort the report point table by the content of the column. Click again to toggle the sort order.

The second row in the report point table is a filter row. All values will be included if the filter setting is blank. To apply a filter, choose a value from the list. The value will be shown in the filter setting and only rows in the table that contain the value in the selected column will be displayed. You can apply multiple filters to the table at the same time. To remove a filter, choose ***All*** from the list of column values in the filter setting.

Copying the report point table

To copy selected contents of the report point table, select cells by dragging with the mouse and press **Ctrl-C** or choose ***Copy*** from the right-click menu. Choose ***Copy Table*** to copy the whole table, as sorted and filtered when applicable. The contents will be copied in tab-separated text format to the Windows clipboard, and can be pasted from there into other programs. All selected cells will be copied as displayed, including header cells and filter settings.

Exporting the report point table

To export the report point table to a tab-separated text file, choose ***File*** →***Export*** →***Report Point Table***. The entire table will be exported, regardless of sorting or filtering.

Report table columns

Columns in the report point table that are not self-explanatory are described below.

Column	Description
File	File number. This column is only shown when the evaluation session includes more than one file, the cycle number is prefixed with a file number. Choose ***File*** →***Properties*** to display the mapping of source files to file numbers.
AbsResp (RU)	Absolute response in RU, calculated as the mean value over the time window.
SD	Standard deviation of data points in the time window, calculated as $SD = \sqrt{\frac{1}{(n-1)}\sum(y-\bar{y})^2}$ where n = number of points and y = response in RU
Slope (RU/s)	Slope during time window in RU s^{-1}, calculated as $slope = \frac{\sum(y-\bar{y})(x-\bar{x})}{\sum(x-\bar{x})^2}$

Column	Description
LRSD	Alignment of slope to a straight line (regression coefficient), calculated as $LRSD = \sqrt{\frac{Q_0}{(n-2)}}$ where $Q_0 = \sum(y-\bar{y})^2 - \frac{(\sum(y-\bar{y})(x-\bar{x}))^2}{\sum(x-\bar{x})^2}$
Keywords	One column is created for each keyword in the data.

9 Result plots

About this chapter

This chapter describes result plot items.

In this chapter

Section		See page
9.1	Creating and editing result plots	116
9.2	Plot display functions	118
9.3	Annotations and comments	120
9.4	Data adjustment functions	123
9.5	Ranking and cut-off	129
9.6	Curve fitting	131

9.1 Creating and editing result plots

Introduction

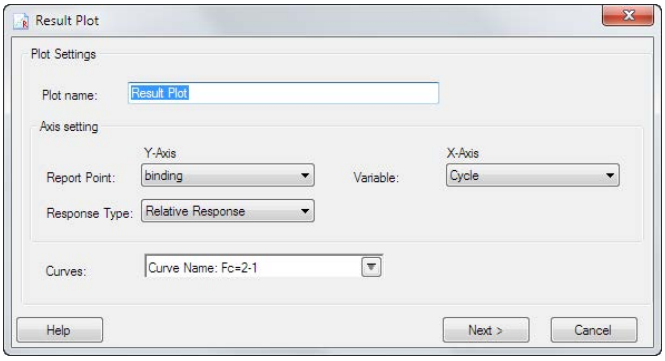
Result plots differ from QC plots in the following respects:

- Result plots support a range of data adjustment and evaluation functions, described in this chapter.
- Excluding points from a result plot affects only the current plot. Other items are not affected.

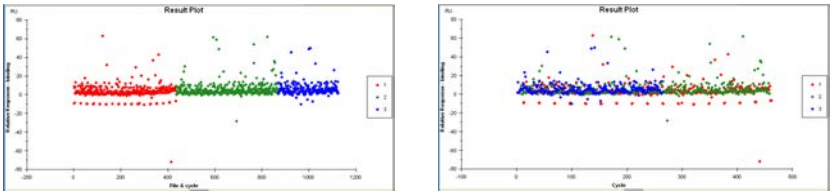
Creating result plots

To create a result plot item, click the ***Evaluation*** →***Result Plot*** button in the navigation panel or choose ***Add Plot*** →***Result Plot*** from the ***Evaluation*** menu. Enter a name for the plot, choose the parameters that define the plot and click ***Next***. Result plots display report point values plotted against a variable: plots of report point against report point are not supported. You can select multiple curves by dragging or using **Ctrl-click** in the curve list to create an overlay plot of points from more than one curve. Cycles that do not contain the selected report point will not be represented in the plot.

Note: *Unlike QC plots, result plots always display all curves selected in the plot definition.*



For evaluation sessions that include multiple result files, the default variable setting is ***File & Cycle***. This will plot the data grouped by file. Choose ***Cycle*** to plot the data by cycle number regardless of file (see illustration below).



*Data from three appended files, plotted against **File & Cycle** (left) and against **Cycle** (right). Points are colored by file.*

Editing result plots

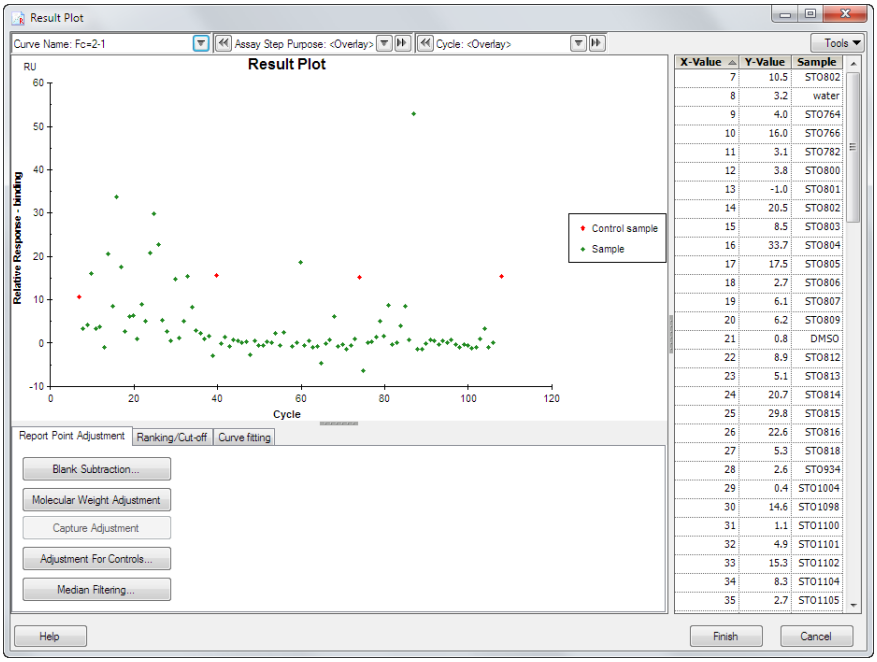
To edit a finished plot, right-click on the item name in the Evaluation Explorer and choose Edit from the menu.

Note: *Editing the plot definition will cancel any adjustments that have been applied.*

9.2 Plot display functions

Presentation

Result plots are displayed with a panel below the plot providing options for data adjustment (see *Section 4.4 Immobilization results, on page 34*). Numerical values for the plotted points are shown in the table to the right.



Selector functions

Selector functions operate as described for QC plots (see *Selecting points for display, on page 108*), with the exception that the curve selection for plots with multiple curves cannot be changed.

Table functions

Table functions operate as described for QC plots (see *Plot table, on page 108*).

Excluding and including points

Points can be excluded from the plot by right-clicking on a point or on one or more selected rows in the table and choosing ***Exclude cycle*** from the right-click menu. Values for excluded points are shown struck out in red text in the table. Use the right-click menu in the table to re-include points.

Note: *Excluding points in a result plot affects the current result plot only. Points that have been excluded in a sensorgram window or QC plot are not shown in result plots and can only be accessed by re-including the points in a sensorgram or QC plot item, then editing the result plot.*

Viewing sensorgrams

To display sensorgrams corresponding to a point, right-click on the point and choose ***Show Sensorgram(s)*** from the right click menu. You can also select multiple rows in the table and right-click on the selection to show multiple sensorgrams in an overlay plot. The sensorgrams are adjusted to zero at the start of the sample injection.

Plot tools

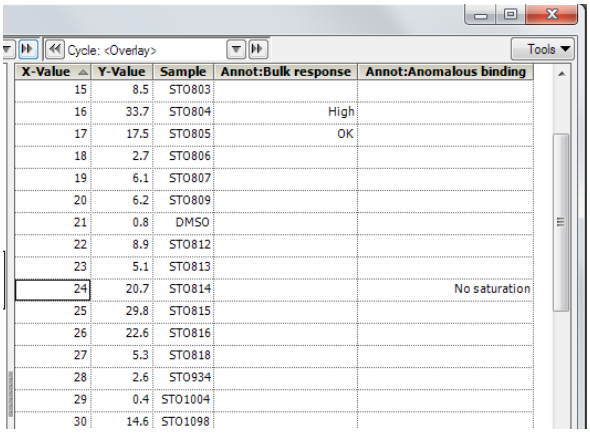
The plot tools ***Color by***, ***Sort*** and ***Table columns*** are available in result plots in the same way as in QC plots (see *Section 8.3 QC plots, on page 107*). In addition, the ***Tools*** menu includes ***Edit Annotations*** (see *Section 9.3 Annotations and comments, on page 120*).

Ranking and cut-off as well as curve fitting functions are provided in a tab on the bottom panel of the plot display (see *Section 9.5 Ranking and cut-off, on page 129*).

9.3 Annotations and comments

Introduction

Samples of particular interest can be marked with annotations that are listed in the result summary table and included in printouts and exported data. Annotations are associated with one or more explanatory comments: in summary tables, comments are listed in a separate column for each annotation.



X-Value	Y-Value	Sample	Annot:Bulk response	Annot:Anomalous binding
15	8.5	STO803		
16	33.7	STO804	High	
17	17.5	STO805	OK	
18	2.7	STO806		
19	6.1	STO807		
20	6.2	STO809		
21	0.8	DMSO		
22	8.9	STO812		
23	5.1	STO813		
24	20.7	STO814		No saturation
25	29.8	STO815		
26	22.6	STO816		
27	5.3	STO818		
28	2.6	STO934		
29	0.4	STO1004		
30	14.6	STO1098		

Scope of annotations

Annotations are available in ***Result Plot***, ***Kinetics*** and ***Affinity*** items. The list of available annotations is common to the three item types, although usage of annotations is local to individual items (so that for example a data point marked with an annotation in one result plot will not automatically be annotated in another). Annotations are applied to data points in result plots and to data series in affinity and kinetic screen.

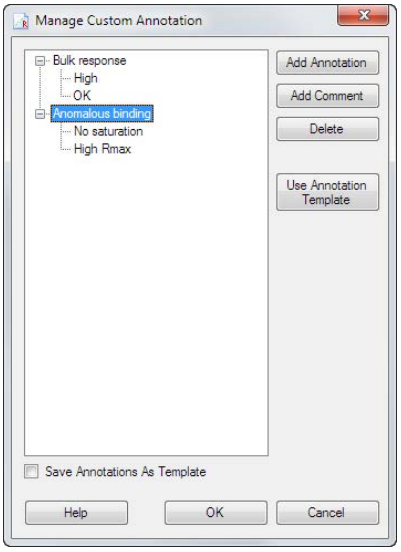
Annotations are saved either locally with the evaluation session or in a global template for use in other evaluation sessions.

- An evaluation session started by opening a result file (.blr) has access to annotations that have been saved as a template.
- A newly created item has access to all annotations in the current evaluation session.
- An item that is edited has access to the annotations existing at the time the item was finished, but not to annotations that were created later.
- A new evaluation session started by opening an evaluation file (.bme) has access to the annotations in the saved session.

Creating and editing annotations

Follow the steps below to create a new annotation.

Step	Action
1	Choose ***Edit Annotations*** from the ***Tools*** menu. *Result:* The ***Manage Annotations*** dialog is displayed.



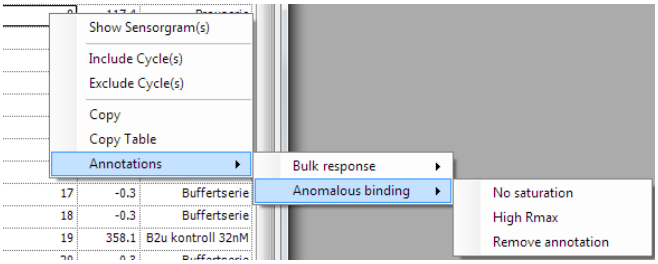
2	Click ***Add Annotation*** and enter the annotation text.
3	Select the annotation, then click ***Add Comment***. Enter a comment text. You can add as many comments as required. ***Note:*** *An annotation must have at least one comment. The annotation text is shown in the column header and the comment in the column content in summary tables.*
4	Check ***Save Annotations As Template*** to make the annotations list available for all evaluation sessions on that specific computer. Any previously saved template will be replaced. If this box is not checked, the list will only be saved with the evaluation file and will not be available for evaluation of other result files.

To edit an annotation or comment text, mark the text in the ***Manage Annotations*** dialog and hold the cursor in the marked text for a couple of seconds until it becomes available for editing. Type the new text. Editing annotations and comments will not affect the text in finished items where the annotation has been used.

Note: *If you change the text of an annotation that has been applied to data points or data series, the column listed for the previous annotation text will be removed from the summary table. Re-display the annotation in the table using the* ***Table columns*** *option.*

Applying and removing annotations

To apply an annotation to a point in a result plot, right-click on the corresponding row in the table and choose ***Annotations*** from the menu. Select multiple rows to apply the same annotation to multiple points. Select the desired annotation and comment from the menu list. Select ***Remove Annotation*** to remove the annotation from the point.



Note: *Annotations are local to the result plot in which they are used and are not transferred to other evaluation items.*

Multiple annotations may be assigned to a single point, but only one comment may be applied for each annotation.

9.4 Data adjustment functions

Introduction

Result plots support the following data adjustment functions:

- Blank subtraction
- Molecular weight adjustment
- Capture adjustment
- Adjustment for controls
- Median filtering

Each of these adjustments is described in detail below. Adjustments must be re-applied if the plot definition is edited: check that the definition is correct before applying adjustments.

Note: *Data adjustments in result plots are applied only to the plot points. Choosing* ***View Sensorgram*** *from the right-click menu on a plot point shows the original sensorgram for the point regardless of adjustments applied in the plot.*

Adjustment dependencies

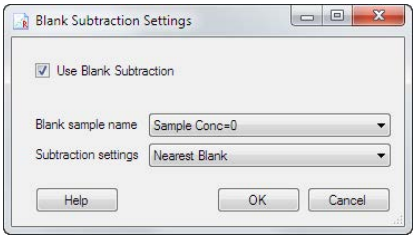
Data adjustments are applied in the order listed above.

The effect of each adjustment may be dependent on the results of adjustments earlier in the sequence (e.g. adjustment for controls will be different according to whether adjustment for molecular weight has been applied or not). If an earlier adjustment is changed, adjustments later in the sequence will be canceled and must be re-applied. Similarly, if changes are made to the data set in the plot by excluding or including points so that an adjustment is affected, the affected and all dependent adjustments will be canceled.

Editing the plot definition will cancel all adjustments and ranking/cut-off boundaries that have been applied.

Blank subtraction

Click ***Blank Subtraction*** to subtract blank contributions from report point values.



Check ***Use Blank Subtraction*** to apply blank subtraction. Choose the sample to be used as blank. Blank samples are either controls or sample cycles with zero concentration. Specify how the blank cycle is chosen:

Setting	Description
Nearest Blank	Nearest blank in the cycle sequence.
Average Nearest Blanks	Average of the nearest preceding and nearest following blanks. If there is no preceding or following blank, the nearest blank is used.
Preceding Blank	Nearest preceding blank in the cycle sequence. If there is no preceding blank, the nearest blank is used.
Following Blank	Nearest following blank in the cycle sequence. If there is no following blank, the nearest blank is used.

Blank values are subtracted from all points in the plot as far as possible, using the same curve and report point settings as the plotted points. For evaluations using appended result files, the blank setting is applied within each file separately.

To remove blank subtraction, click ***Blank Subtraction*** and uncheck ***Use Blank Subtraction***.

Molecular weight adjustment

Adjustment for molecular weight divides the response by the molecular weight of the analyte and displays the result multiplied by 100. Use this function to normalize the response from differently sized analytes so that the values reflect molar binding levels rather than weight-based levels.

Any points for which analyte molecular weight is missing from the keyword table or is entered as zero will be excluded from the adjusted plot.

Capture adjustment

Capture adjustment corrects sample responses for variations in the levels of captured ligand between cycles by dividing the sample response with the response for captured ligand. When capture adjustment has been applied, adjusted response levels will be expressed as sample response divided by capture level.

Note: *Capture adjustment relies on response values from the report points* ***capture_baseline*** *and* ***baseline****. The adjustment cannot be applied if these report points are not present.*

Capture adjustment should only be applied to report points that represent analyte response (either direct or enhanced). The adjustment is not appropriate for report points placed before the sample injection.

Adjustment for controls

Adjustment for controls compensates for systematic changes in response during the course of the assay, such as progressive loss of binding capacity. Adjustment for controls can also be used to normalize response levels from multiple files that are evaluated together, provided that the same controls are used in all files. Adjustment is calculated from the response values obtained for control samples analyzed at intervals during the assay.

Adjustment normalizes the sample responses relative to the positive and negative control levels as follows. Start-up cycles are automatically excluded from the adjustment calculations.

- Curves are fitted to the control sample responses for positive and (if used) negative controls. If no negative control is specified, the negative control level will be set to zero. The ***Linear*** option fits the points to a function with the form $y = ax + b$ (where a and b are constants). ***Polynomial*** fits the points to a second-degree function with the form $y = ax^2 + bx + c$ (where a, b and c are constants).
- The fitted line(s) are transformed to straight horizontal lines with values 100 for the positive control and 0 for the negative control.
- The transformation used to create straight horizontal lines for the control points is applied to all points in the plot (including the actual control sample responses). Each point retains the same position relative to the positive and negative controls before and after adjustment.

Adjustment for controls cannot be applied in regions where the positive control curve lies below the negative control level. Any points that lie in such regions will be excluded from the adjusted plot.

For evaluation sessions that include multiple result files, adjustment for controls is applied separately to each file, regardless of whether the data is plotted against ***Cycle*** or ***File & Cycle***. However, if the same control is used in all files, applying adjustment for controls will normalize the response levels between files.

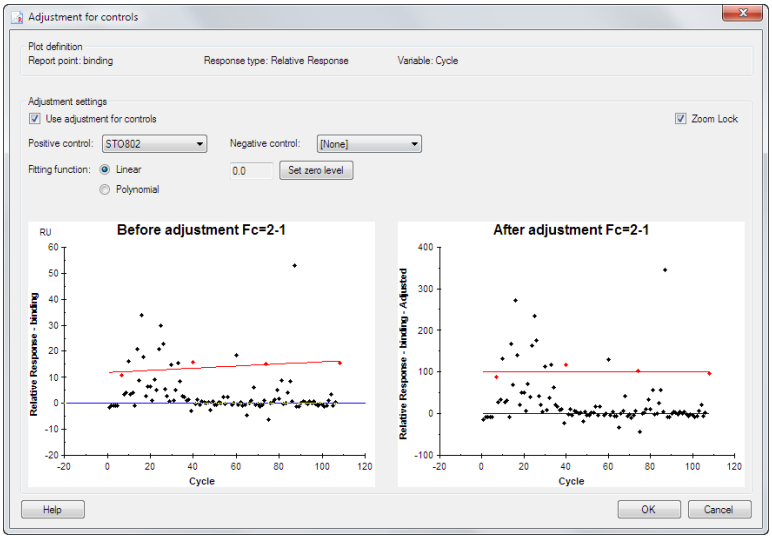
Follow the steps below to apply adjustment for controls.

Step	Action
1	Choose ***Adjustment for controls***.
2	Check ***Use adjustment for controls***.
3	Select the sample to use as a positive control. You can also select a sample or specify a response level for the negative control.
4	Select whether the adjustment should be made using a linear or polynomial fitting function.

Step	Action

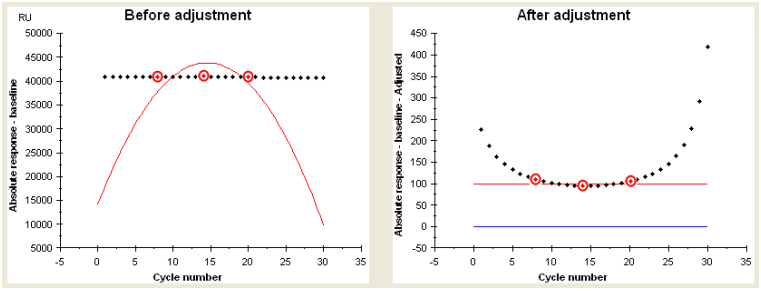
Result:

The display panels in the dialog show a plot of the response against cycle number before and after adjustment.



5 Click ***OK*** to apply the adjustment.

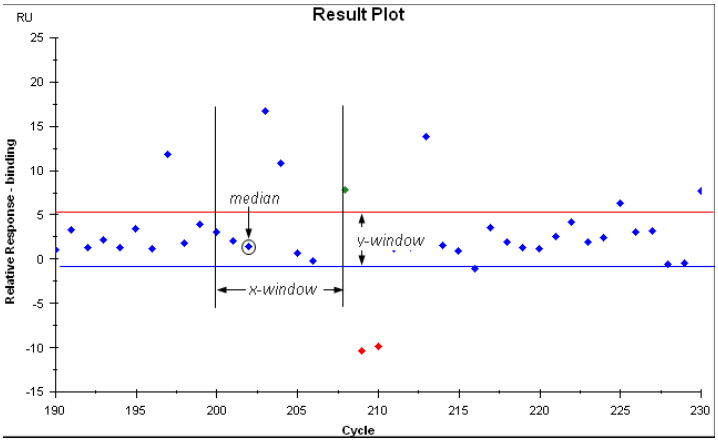
Note: *Beware of using a polynomial fitting function with less than 4 control samples. The parabolic curve created by the function can deviate greatly from the points, leading to adjustment that does not reflect the drift in the control responses (see illustration below).*



Median filtering

Applying a median filter to a plot can markedly reduce noise and eliminate drift without obscuring binding responses. The filter is most useful for plots with drift or periodic variation in the baseline, and a y-axis window can be set so that the window includes presumed non-binders and excludes most of the potential binders. The effect of the filter is to align points within the y-axis window to a new zero baseline. Positive control samples should be included at regular intervals in the run, to provide a check on the suitability of the median filter settings. The filter is applied using the set y-axis (response) window and a sliding x-axis (cycle) window as follows. Start-up cycles are automatically excluded from the median filtering.

- The median value for each x-coordinate is calculated from the points in the region defined by the x- and y-axis windows. Where points fall outside the y-axis window, more cycles are included so that the number of points within the windows is constant.
- Each point in the plot (including those that lie outside the y-axis window) is adjusted by subtracting the median value. A new baseline is thereby defined with response value 0.



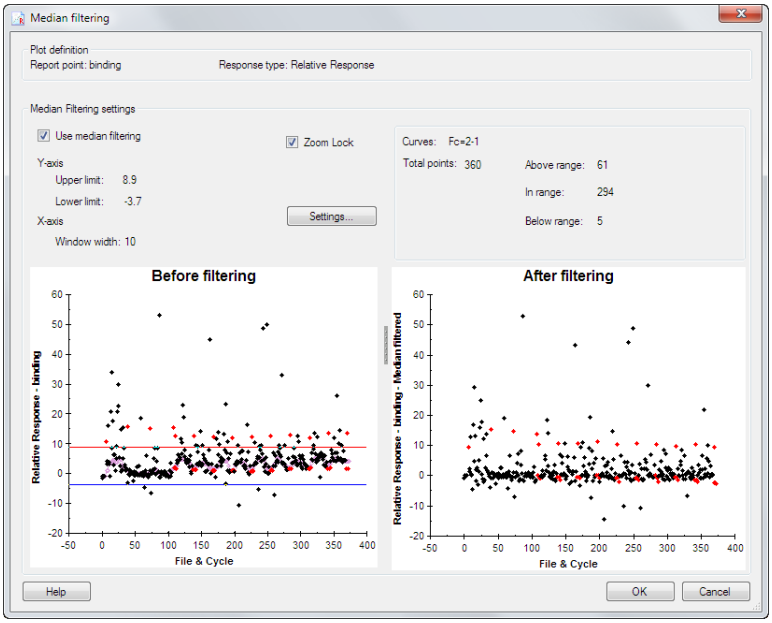
Principle of median filtering. The median response value of the points within the x- and y-windows is used as a new baseline for the current cycle.

For evaluation sessions that include multiple result files, median filter is applied to data grouped by file, regardless of whether the data is plotted against ***Cycle*** or ***File & Cycle***.

Note: *When adjacent files in a multi-file session have significantly different response levels, the order of the files will affect the details of the border effects. This can often be avoided by using adjustment for controls to normalize the response values (Section 4.4.5). To ensure that files are appended in the required order, append the files one by one to the evaluation session. You can see the order of the files in* ***File →Properties****.*

Follow the steps below to apply a median filter.

Step	Action
1	Choose ***Median filter***.
2	Check ***Use median filtering***.



3	To set the y-axis window, either drag the horizontal lines or click ***Settings*** and enter upper and lower response limits. The window should be wide enough to cover noise variations in negative (non-binding) samples, but narrow enough to exclude the response from binders as far as possible.
4	To set the x-axis window, click ***Settings*** and enter the number of points in the window (minimum 5).
5	Click ***OK*** to apply the filter.

Judge the appropriateness of the median filter settings with reference to the positive control responses. Filtering should not introduce new trends or significantly increase scatter among the positive control points.

Note: *Choosing appropriate settings can be critical to the value of median filtering. It may be necessary to experiment with the window settings to obtain the best results for a given plot. Using inappropriate settings or applying median filter to plots for which it is not suited can introduce artefacts in the evaluation.*

9.5 Ranking and cut-off

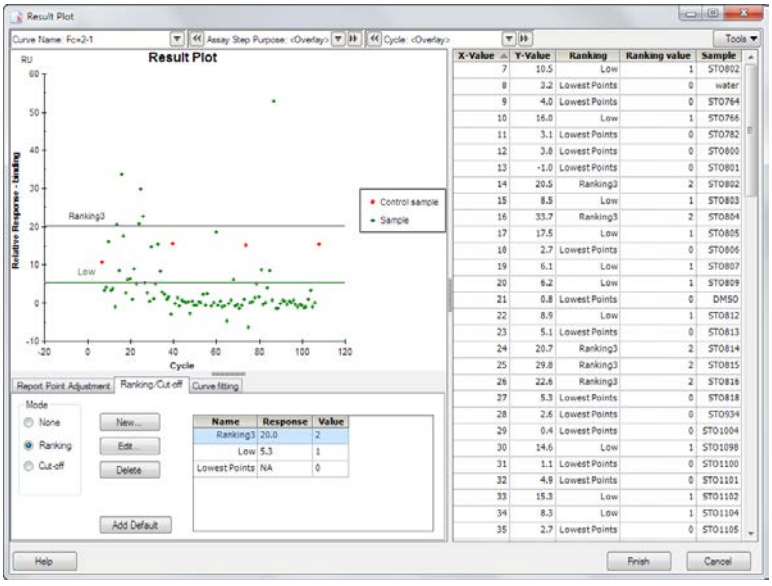
Ranking boundaries

Ranking boundaries divide the plot into a user-defined number of response regions. Ranking boundaries are set automatically on the basis of average responses for control samples and may be edited freely. Custom boundaries can also be added.

Points are ranked in the plot table according to the name value of the nearest lower boundary. Points below the lowest boundary are ranked as ***Lowest points***. Boundaries are also assigned a numerical value that can be useful in data processing in third-party software.

Follow the steps below to manage ranking boundaries.

Step	Action
1	Choose ***Ranking*** on the ***Ranking/Cut-off*** tab.



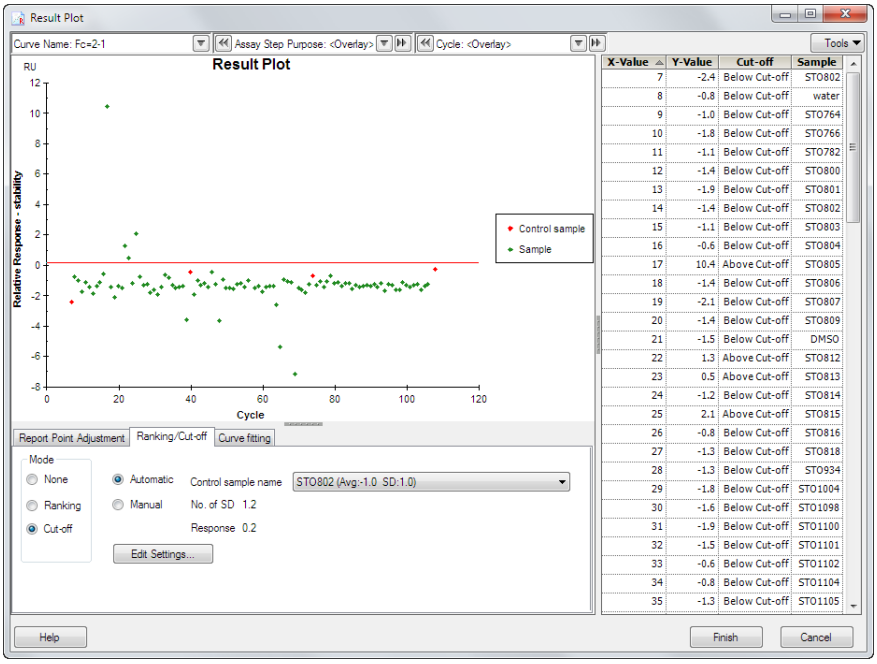
2	To add a new boundary, click ***New*** and enter the settings for the boundary.
3	To edit a boundary, drag the boundary line in the plot or click on ***Edit*** and enter new settings.
4	To delete a boundary, select the boundary and click ***Delete***.
5	Click ***Add Default*** to add boundaries at average response levels for control samples. Existing boundaries will not be affected.

Note: *Ranking will be turned off if the plot definition is edited.*

Cut-off boundaries

Cut-off boundaries divide the plot into two horizontal regions, above and below a cut-off line. Points are classed as either ***Above Cut-off*** or ***Below Cut-off*** depending on the exact response value.

Note: *Points that appear to lie precisely on the cut-off boundary may be classed as either* ***Above Cut-off*** *or* ***Below Cut-off*** *depending on the exact response value at a precision higher than that displayed in the software.*



In ***Automatic*** mode, the boundary is set on the basis of the average response of a control sample plus a specified number of SD (default 3). Choose the control sample series to use for the boundary. Click ***Edit Settings*** to change the number of SD. You can also change the response level by dragging the boundary line or entering a new level in the ***Edit Settings*** dialog. The number of SD displayed is updated to reflect the changed setting relative to the selected control sample series.

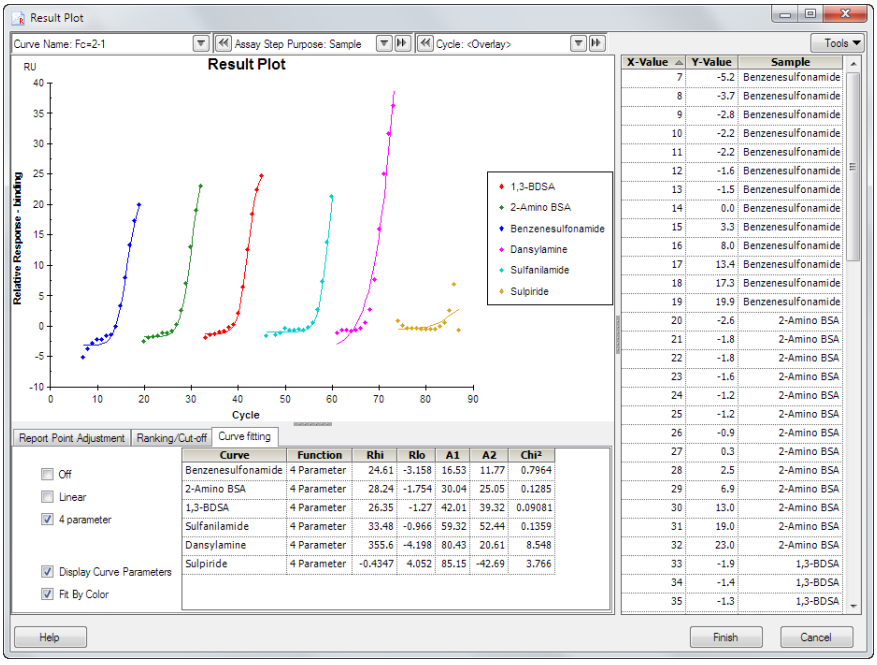
In ***Manual*** mode, the boundary is set by dragging the boundary in the plot or entering the response level in the ***Edit Settings*** dialog.

9.6 Curve fitting

Fitting curves to points

The ***Curve Fitting*** tab allows you to fit lines to the points in the plot. using either linear or curved (4-parameter) fitting functions. If ***Fit by color*** is checked, each color will be fitted to an independent line. If this option is not checked, all points derived from the same curve will be fitted to a single line. Check ***Display Curve Parameters*** to show the parameter values for the fitted curves.

Curve fitting can only be applied to plots that are sorted ***As defined***.



Curve	Function	Rhi	Rlo	A1	A2	Chi²
Benzenesulfonamide	4 Parameter	24.61	-3.158	16.53	11.77	0.7964
2-Amino BSA	4 Parameter	28.24	-1.754	30.04	25.05	0.1285
1,3-BDSA	4 Parameter	26.35	-1.27	42.01	39.32	0.09081
Sulfanilamide	4 Parameter	33.48	-0.966	59.32	52.44	0.1359
Dansylamine	4 Parameter	355.6	-4.198	80.43	20.61	8.548
Sulpiride	4 Parameter	-0.4347	4.052	85.15	-42.69	3.766

X-Value	Y-Value	Sample
7	-5.2	Benzenesulfonamide
8	-3.7	Benzenesulfonamide
9	-2.8	Benzenesulfonamide
10	-2.2	Benzenesulfonamide
11	-2.2	Benzenesulfonamide
12	-1.6	Benzenesulfonamide
13	-1.5	Benzenesulfonamide
14	0.0	Benzenesulfonamide
15	3.3	Benzenesulfonamide
16	8.0	Benzenesulfonamide
17	13.4	Benzenesulfonamide
18	17.3	Benzenesulfonamide
19	19.9	Benzenesulfonamide
20	-2.6	2-Amino BSA
21	-1.8	2-Amino BSA
22	-1.8	2-Amino BSA
23	-1.6	2-Amino BSA
24	-1.2	2-Amino BSA
25	-1.2	2-Amino BSA
26	-0.9	2-Amino BSA
27	0.3	2-Amino BSA
28	2.5	2-Amino BSA
29	6.9	2-Amino BSA
30	13.0	2-Amino BSA
31	19.0	2-Amino BSA
32	23.0	2-Amino BSA
33	-1.9	1,3-BDSA
34	-1.4	1,3-BDSA
35	-1.3	1,3-BDSA

Fitting functions

Function	Equation
Linear fit	$y = \text{slope} * x + \text{intercept}$

Function	Equation
4-parameter fit	$y = {}_{I}R_{hi} - \frac{R_{hi} - R_{lo}}{1 + \left(\frac{x}{A_1}\right)^{A_2}}$

Parameter	Description
R_{hi} and R_{lo}	Fitting parameters that correspond to the maximum and minimum response levels respectively.
A_1 and A_2	Additional fitting parameters.

10 Fragment screening evaluation

About this chapter

Fragment screening or fragment-based drug discovery (FBDD) refers to screening of fragment libraries for binding to potential drug targets. Biacore S200 Evaluation Software provides two evaluation functions that are specifically designed for evaluation of fragment screening assays where binding is analyzed at a single concentration of each fragment.

Function	Description	Refer to
Clean Screen	Assists in identifying compounds in the fragment library that show persistent or residual binding to a degree that can cause problems in subsequent steps.	See *Section 10.1 Clean screen, on page 134*.
Binding Level Screen	Used to identify promising fragments on the basis of binding level and binding behavior, with the aim of reducing the number of compounds carried forward to more work-intensive steps.	See *Section 10.2 Binding level screen, on page 138*.

In this chapter

Section		See page
10.1	Clean screen	134
10.2	Binding level screen	138

10.1 Clean screen

Requirements

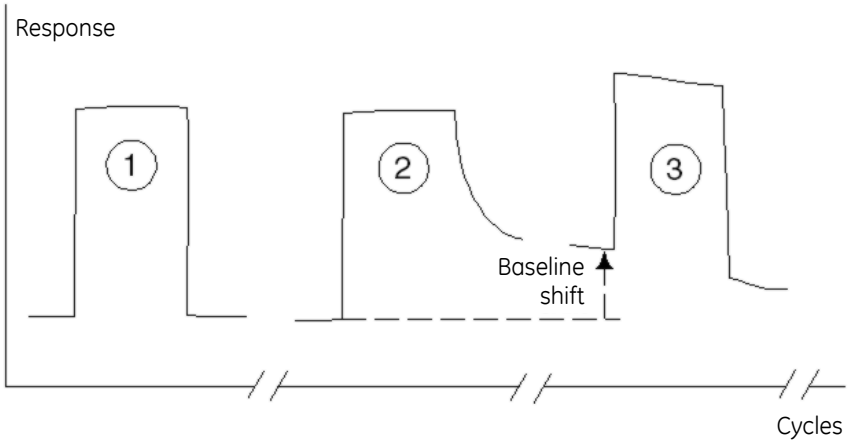
There are no special requirements for fragment clean screen evaluation. The evaluation uses baseline report points which are created by default.

The evaluation is based on measurement of baseline shifts between cycles. The last cycle is evaluated from the response shift between the baseline and the end of the cycle.

Note: *All cycles except **Startup** cycles are included in the **Clean Screen** evaluation.*

Principle

Clean Screen evaluation is based on shifts in baseline response between one cycle and the next, as an indicator of residual or persistent binding. Samples that show persistent binding to the target will be identified by a significant positive shift in the baseline. For the last cycle, the shift is measured as the response difference between the baseline and the end of the cycle.

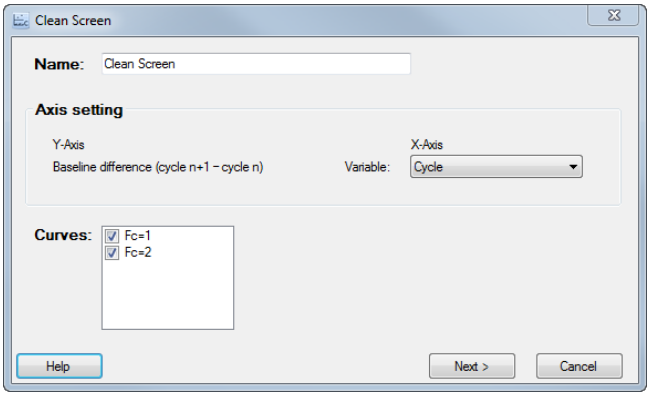


Principle of clean screen for identifying fragments with persistent binding. Cycle 1 shows a binding profile typical for many fragments, with rapid association and dissociation. The fragment in cycle 2 shows persistent binding, evident from the slow dissociation, with the result that response from cycle 2 is carried over into cycle 3.

Procedure

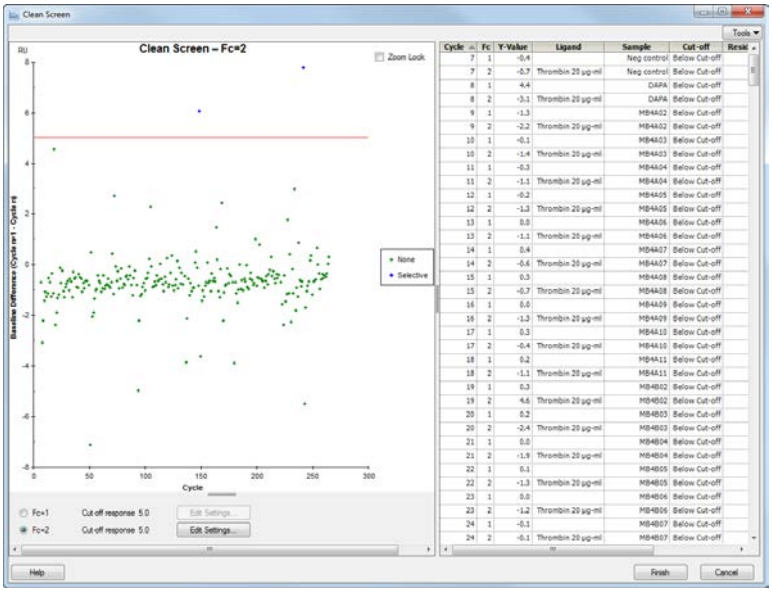
Follow the steps below to perform a ***Clean Screen*** evaluation.

Step	Action
1	Open the required result file(s).
2	Click ***Clean Screen*** in the navigator panel. *Result:* The ***Clean Screen*** preparation dialog opens.



3	Enter a name for the ***Clean Screen*** item if required.
4	Choose the x-axis for the ***Clean Screen*** plot. The default setting is ***Cycle***: available alternatives are ***Conc*** and ***MW***. The y-axis always shows the baseline difference between the current and next cycle.
5	Select the curves to include in the item. Active and reference curves may be selected, but reference-subtracted curves are not available.
6	Click ***Next***.

Step	Action
	Result: The results are displayed with a default cut-off setting of 5 RU.



Use the options under the ***Tools*** button to change settings for the plot and table display.

7 If you have included more than one curve in the evaluation, choose the curve to display in the left-hand (plot) panel.

Note:

The table in the right-hand panel always lists results for all included curves.

8 Click ***Edit Settings*** or drag the cut-off boundary if you want to adjust the boundary position.

9 Click ***Finish*** to finalize the evaluation.

Results

Residual Binding is derived from consideration of the cut-off value for all curves in the evaluation, and can have one of three values:

Value	Meaning
None	The baseline difference is below the cut-off for all curves.
Selective	The baseline difference is above the cut-off for some but not all curves.
General	The baseline difference is above the cut-off for all curves.

Plot points are by default colored by ***Residual Binding*** value.

Click on the table column header for ***Cut-off*** or ***Residual Binding*** to sort the table by these values and assist identification of samples that give residual binding.

Click or hover on a point in the plot to identify the sample to which the point refers. Right-click on a point in the plot or a row in the table for options to display the sensorgram, to assist in judging whether the compound should be excluded from further screening or not. Select one or more rows in the table to identify the corresponding point(s) in the plot.

10.2 Binding level screen

Principle

Binding Level Screen for FBDD helps to prioritize and select fragments for further development based on binding levels at a single fragment concentration. In this respect, the principle of the evaluation is similar to binding level analysis for other compounds using result plots (see *Chapter 9 Result plots, on page 115*). However, the dedicated ***Binding Level Screen*** evaluation item includes assessment of binding behavior (see below) and supports selection of candidates for further processing based on the number of candidates (or the fraction of the screened fragment library) as well as the actual binding levels.

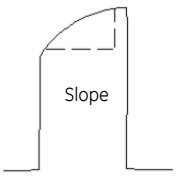
Requirements

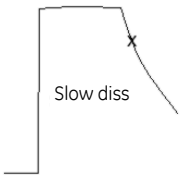
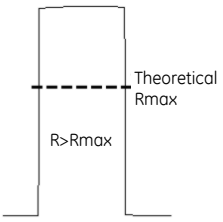
Requirements for ***Binding Level Screen*** evaluation are listed below

- The evaluation can only be applied to solvent-corrected data (see *Section 7.3 Solvent correction, on page 92*).
- A report point named ***binding_early***, placed shortly after the start of the sample injection, is recommended but not mandatory.
- If the binding behavior indicator ***R>Rmax*** is to be used (see *Binding behavior indicators, on page 138*), the ligand molecular weight must be provided, either entered during the immobilization or added to ***Chip Information*** through the keyword table (see *Ligand details, on page 87*).

Binding behavior indicators

Atypical binding behavior is relatively common in fragment screening work, and is identified in binding level screen evaluation by three independent criteria.

Name	Description	Sensorgram appearance
Slope	Indicates a significantly increasing response during sample injection instead of the normally expected rapid binding to a steady-state level. ***Slope*** is determined from the average slope of the sensorgram between the report points ***binding_early*** (shortly after injection start) and ***binding*** (shortly before injection end).	

Name	Description	Sensorgram appearance
Slow diss	Indicates that the compound does not dissociate immediately after the end of the sample injection. ***Slow diss*** is determined from the relative response above baseline for the report point ***stability*** (shortly after the end of the injection).	
R>Rmax	Indicates that the maximum response reached during sample injection is higher than expected for the ligand immobilization level. ***R>Rmax*** is determined from the highest response during sample injection and the theoretical maximum binding capacity based on 1:1 binding. Calculation of the theoretical maximum binding capacity requires the molecular weight of the ligand, provided either during immobilization or edited in the ***Chip Information***. This parameter is not supported for screens using captured ligands.	

Samples with more than one behavior indicator markers are listed as ***Multiple*** in the screening results.

Procedure

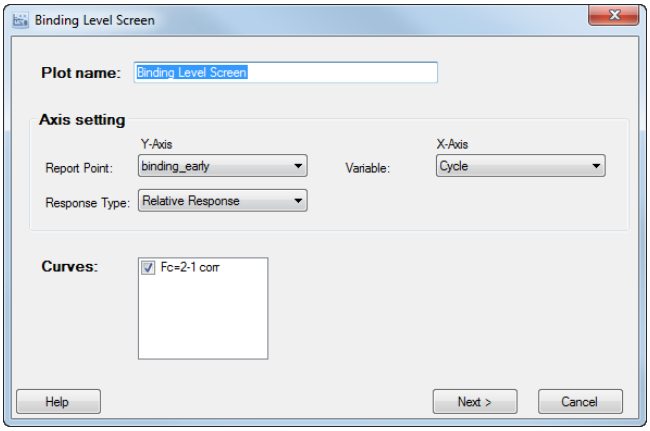
Follow the steps below to perform a ***Binding Level Screen*** evaluation.

Step	Action
1	Open the required result file(s).
2	Apply solvent correction (see *Section 7.3 Solvent correction, on page 92*). Binding level screen evaluation can only be applied to corrected curves.
3	Click ***Binding Level Screen*** in the navigator panel.

Step	Action

Result:

The ***Binding Level Screen*** preparation dialog opens.



4 Enter a name for the ***Binding Level Screen*** item if required.

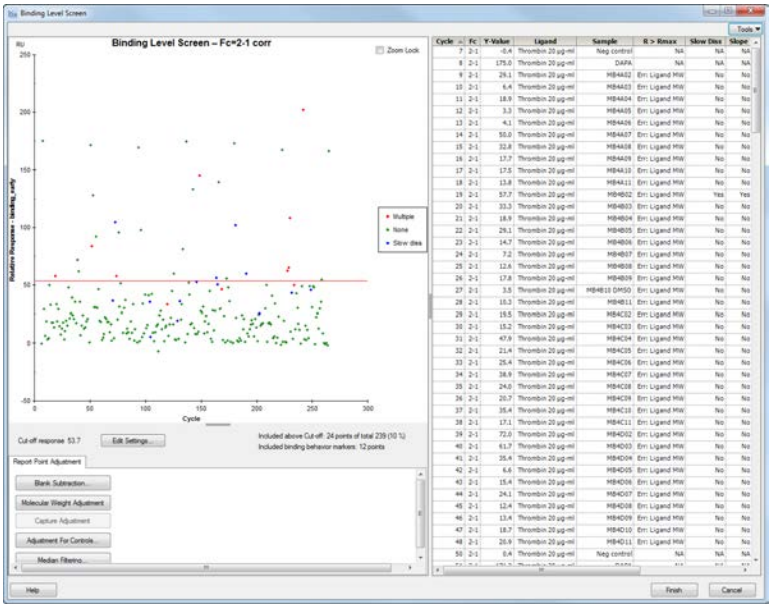
5 Specify the settings for the plot:

- **y-axis:** recommended report point ***binding_early***, response type ***Relative Response***.
- **x-axis:** default ***Cycle***, alternatives ***Conc*** and ***MW***.

6 Select the curves to include in the item. Only reference-subtracted and corrected curves are available.

7 Click ***Next***.

Step	Action
	Result: The binding level screen plot is displayed, with a default cut-off set to the average response for the selected control sample plus 3 standard deviations (SD). If the data includes more than one control sample, the default setting is calculated from the first control in alphabetical order.



Use the options under the ***Tools*** button to change settings for the plot and table display.

Note:

No report point adjustments are applied in the initial results display. Do not accept the results before applying adjustments.

8 Apply report point adjustments as required (see *Section 9.4 Data adjustment functions, on page 123*).

9 Click ***Edit Settings*** or drag the cut-off boundary if you want to adjust the boundary position.

10 Click ***Finish*** to finalize the evaluation.

11 Evaluating kinetics and affinity

About this chapter

This chapter describes how to evaluate kinetics and affinity analyses.

In this chapter

Section		See page
11.1	Requirements and recommendations	143
11.2	Presentation	144
11.3	Evaluation procedure	148
11.4	Dealing with low affinity interactions	158
11.5	Annotations and comments	162
11.6	Exporting kinetics and affinity results	163
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11.1 Requirements and recommendations

Requirements

- Experiments using low molecular weight analytes in buffers containing DMSO should include solvent correction cycles, although this is not directly required for the evaluation procedure.
- At least one assay step is required with purpose ***Sample***, connected to a cycle type that includes one ***Sample*** injection. Only data from the ***Sample*** injection in assay steps with purpose ***Sample*** will be used for evaluation.
- Sample concentration must be specified in the variable ***Conc***. If weight-based units are used, a molecular weight for the analyte must be specified in the variable ***MW***.
- Steady state affinity evaluation requires at least three unique non-zero concentrations.

Assay development

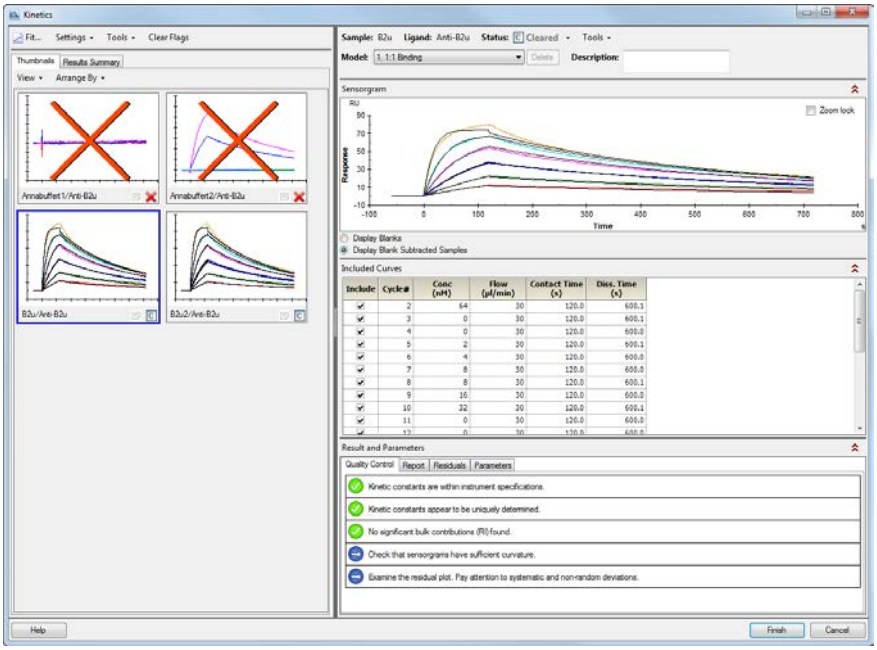
Before attempting to determine the kinetics or affinity for a previously uncharacterized interaction, run an experiment using the pre-defined ***Interaction characteristics*** method template in the ***Assay Development*** folder. At this stage, run 5 sample injections covering a 100-fold concentration range. Repeat the test if necessary with adjusted sample concentrations and/or injection times. This should give a preliminary indication of suitable injection times and sample concentrations for more thorough analysis.

Recommendations

Default recommendations for kinetics determinations are a concentration series with three non-zero analyte concentrations. For single-cycle kinetics, run one blank cycle with zero concentration. For single-cycle kinetics, run one blank cycle with the same injection series as the sample, with buffer replacing the sample for each injection.

11.2 Presentation

Screen panels



The left-hand panel contains tabs for thumbnails and results summary. The right-hand panel contains a detailed view of the currently selected data series. Selected data series are marked with a blue border.

Thumbnails

Thumbnail size

Choose the thumbnail display size from the ***View*** menu on the ***Thumbnails*** tab.

Option	Description
Small	Provides an overview of many thumbnails for general comparison. Details will not be legible in the thumbnails. Individual thumbnails are identified in a tool tip.
Standard	Shows the thumbnails with identification of the ligand and analyte. Axis scales are not marked.
Extended	Shows more detailed identification of each thumbnail with fitting model and rate or affinity constants. Axis scales are marked.

Thumbnail content

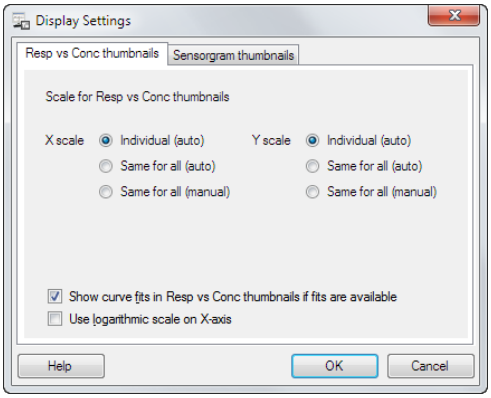
By default, thumbnails show sensorgrams for kinetic screen or a plot of response against concentration for affinity screen. Sensorgrams are blank-subtracted and adjusted to t=0 at injection start.

Choose between these alternatives from the ***View*** menu on the ***Thumbnails*** tab.

Note: *Both options are available in both* ***Kinetics*** *and* ***Affinity*** *evaluations. However, affinity evaluation cannot be performed in a* ***Kinetics*** *item and vice versa.*

Thumbnail display settings

Choose ***Display Settings*** from the ***View*** to change thumbnail display settings.



When the same x- and/or y-axis scales are chosen, the scale(s) will be set to include the widest range in the currently included set of thumbnails. Using individual scaling will scale each thumbnail according to the range of data in the thumbnail.

Check ***Show curve fits...*** to display the fitted curves overlaid on the experimental data in the thumbnails. This may not have any significant effect on the appearance of small thumbnails for kinetic evaluation items, but will in general be readily visible for affinity items.

The Use logarithmic scale on x-axis option is only appropriate for affinity items.

Sorting thumbnails

Choose a parameter in the ***Arrange by*** menu on the ***Thumbnails*** tab to sort the thumbnails. The available parameters correspond to the table columns on the ***Results Summary*** tab.

Sorting the thumbnail display also sorts the table on the ***Results Summary*** tab and *vice versa*.

Note: *Thumbnails are sorted by default in ascending order. Options for ascending and descending order appear in the menu once the thumbnails have been sorted.*

Results summary

The ***Results Summary*** tab summarizes the results. Before evaluation, the tab shows the available information for the data series. Results are shown after fitting has been performed.

The graphical display on the ***Results Summary*** tab shows a KD plot for affinity evalulation and an on-off rate map for kinetics evaluation (see *Results summary plots, on page 156*).

Note: *The **Results Summary** table includes columns for **Evaluation File** and **Image File**. These columns are completed when the file is saved and data is exported using **Export All Graphs and Table** respectively (see Appendix A.1 Exporting data, on page 194 for details of export functions).*

Details

The right-hand panel shows the details of the currently selected series in up to four sub-panels. Click ⌄⌄ and ⌃⌃ to expand and collapse the sub-panels respectively.

Illustration	Description
Response-Concentration Plot (Affinity evaluation only)	This sub-panel shows the fitted data for affinity items. A vertical line indicates the calculated K_D value. The line is red if the calculated K_D is more than half the maximum sample concentration used.
Sensorgram	This sub-panel displays the sensorgrams used in the evaluation. Choose ***Display Blanks*** to show blank sensorgrams instead of sample sensorgrams. For kinetic items, the fitted curves are shown in black overlaid on the sample data.
Included Curves	This sub-panel lists the curves in the data series. Use the ***Include*** checkbox to include or exclude sample curves and blanks from the series.
Results and Parameters	This sub-panel lists the fitting report and the parameters used. Kinetic evaluation using the 1:1 model also generates a QC report (see *Section 12.3 Quality control tab, on page 171*).

You can enter a description for the fitting at the top of the details panel.

If you have performed several fittings for a data series, choose which fitting to display from the ***Model*** list. The chosen fitting will be shown in both the detail panel and the thumbnail for the data series.

11.3 Evaluation procedure

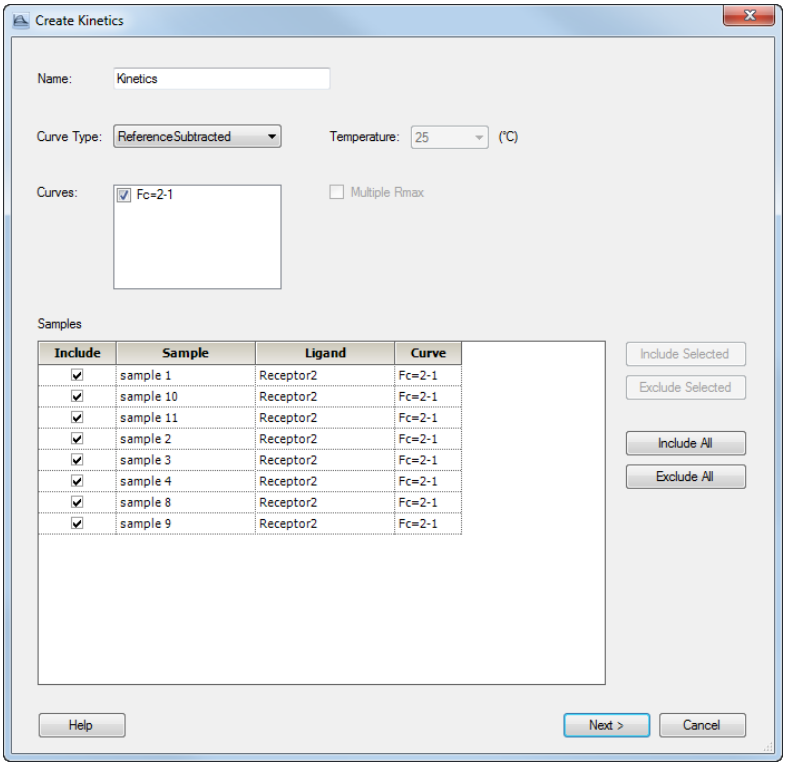
Introduction

This section describes how to perform a kinetics or affinity evaluation. Steps are the same for both kinetics and affinity unless otherwise stated.

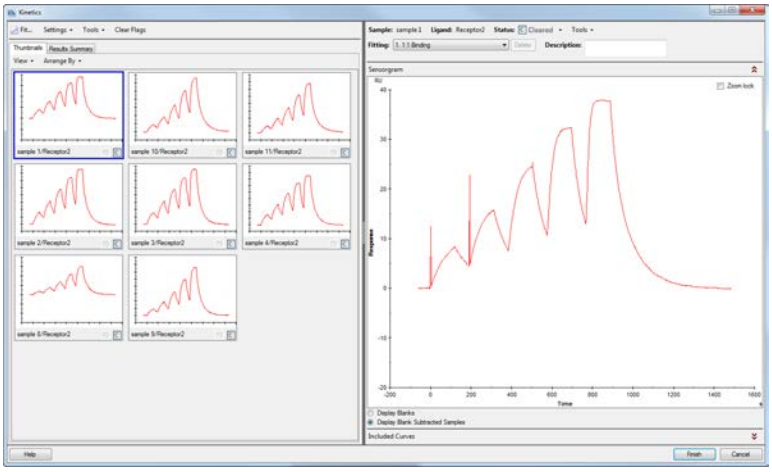
Create kinetics and affinity evaluation

Follow the steps below to create a kinetics or affinity evaluation.

Step	Action
1	Click the ***Kinetics*** or ***Affinity*** button in the navigator panel to start a ***Kinetics*** or ***Affinity*** evaluation respectively. *Result:* The ***Create...*** screen is displayed, with a list of sample series in the result file.



Step	Action
	A sample series is defined as a set of cycles with the same analyte and ligand name and the same analysis temperature. For evaluation of appended result files, cycles in different files form separate series.
2	Provide a name for the item if desired.
3	Choose the curve type and select the curves to be included in the evaluation item.
4	Check ***Multiple Rmax*** to combine sample series with different ***Curve*** settings, allowing global evaluation of series with different ligand densities. If the option is not checked, series are separated by ***Curve***.
5	If the result file contains measurements at different temperatures, choose the temperature to evaluate.
6	Click ***Next***. Result: The main evaluation interface is displayed.



Series status

The status of each series can be set in the main evaluation interface by using either the symbol below each thumbnail or the status indicator at the top of the detail view panel. You can also set the status for one or more series in the thumbnail display or results summary table, using the right-click option ***Set Selection to***. The status can be changed both before and after evaluation.

Icon	Status	Description
✖	Rejected	Cannot be addressed with fit settings or fitting. Can be hidden.

Icon	Status	Description
	Cleared	Default setting. Can be addressed with fit settings and fitting.
	Flagged	Can be used to select series for fit settings and fitting.
	Accepted	Can only be set after fitting. Cannot be addressed with fit settings or fitting.

Status settings remain in force until changed by the user. Click ***Clear flags*** to set all flagged series to ***Cleared***.

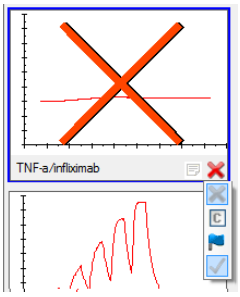
You can hide rejected series by turning on the ***Tools*** →***Hide rejected*** option in the thumbnail panel.

Fit settings and fitting can be applied to series on the basis of selection and/or status.

Preliminary examination and adjustment

An overview of the screening is provided by the thumbnails in the left-hand panel, with one thumbnail for each data series.

Scan through the thumbnails and set the status to ***Rejected*** for series that are clearly disturbed or unsuitable for evaluation. Rejected series are marked with a red X on the thumbnail, and can be hidden using the ***Tools*** →***Hide rejected*** option in the thumbnail panel.



For closer examination of the data, select a thumbnail to display the series in the detail view in the right-hand panel.

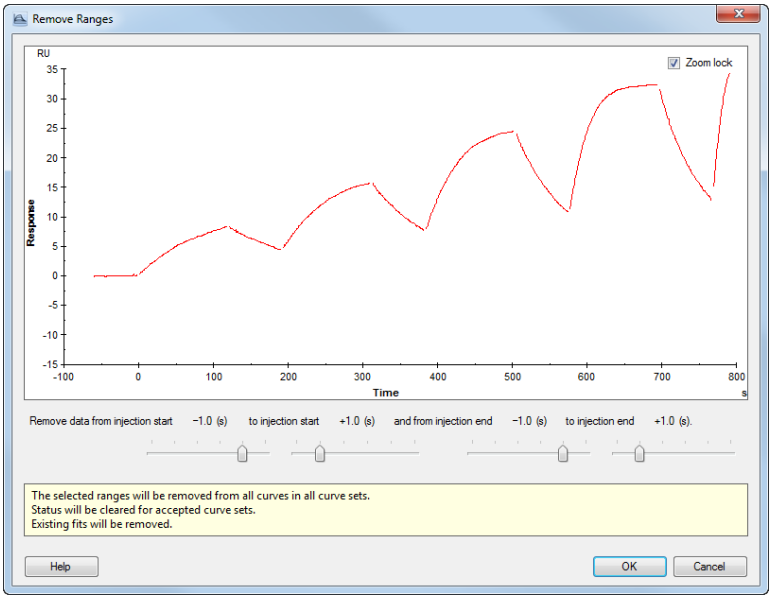
Sample sensorgrams are displayed in the detail panel by default, corrected by subtracting an average of the blank (zero-concentration) sensorgrams. Choose ***Display blanks*** to examine the blank sensorgrams.

The ***Included Curves*** list shows both the sample series (included by default) and blanks from other sample series (excluded by default). Use the ***Include*** check-mark in this list to define which curves are included in the data series.

Removing injection disturbances from all sensorgrams

Follow the steps below to remove data at the beginning and end of the sample injection, to avoid disturbances associated with injection start and stop.

Step	Action
1	Choose ***Tools*** →***Remove Ranges*** in the thumbnail panel. *Result:* The ***Remove Ranges*** dialog is displayed.

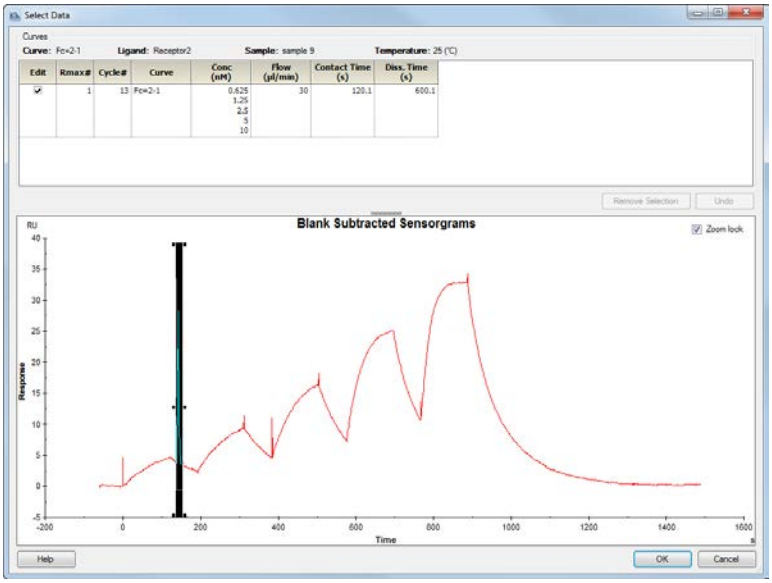


2	Adjust the sliders as required. You can remove up to ±5 s at the beginning and end of the sample injection(s). *Result:* The ranges are removed from all sensorgrams in all series. Any existing fitting results will be removed from the item.
3	Click ***OK***. Check that the range settings are appropriate for all series. Repeat the adjustment if necessary.

Removing disturbances from individual sensorgrams

Follow the steps below to remove disturbed data from individual sensorgrams. This operation can be applied to any part of the sensorgram.

Step	Action
1	Choose ***Tools*** →***Select data*** in the detail panel. *Result:* The ***Select data*** dialog is displayed.



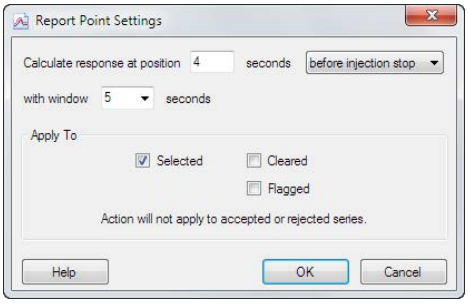
2	Remove the checkmark from the ***Edit*** column in the curve table for the curves that are to be left unchanged. All curves are selected by default and are shown in dark color. Curves that are not selected for editing are shown in light gray. ***Note:*** *All curves will be evaluated, whether they are selected for editing or not. Removing the **Edit** checkmark does not exclude a curve from the data set for evaluation.*
3	Select the region to be edited by dragging with the right mouse button.
4	Click ***Remove Selection***.
5	Click ***Undo*** if you want to restore the deleted data.

Note: *Brief transient disturbances usually have negligible effect on kinetic results, but you should beware of removing disturbances from the report point window for affinity evaluation. If the report point is affected by disturbances, move the report point instead (see below).*

Moving the report point for affinity evaluation

Steady state binding levels for affinity screen are calculated from a report point placed by default 4 seconds before the end of the sample injection. Follow the steps below to move this report point.

Note: *This report point is marked on the sensorgrams in the detail view but is not listed in the report point table. You cannot use a report point from the report point table for this purpose.*

Step	Action
1	Choose ***Settings*** →***Report Point Settings*** in the thumbnail panel. *Result:* The ***Report Point Settings*** dialog is displayed. 
2	Change the position and window as required.
3	Select the series to which the change should be applied (***Selected***/***Cleared***/***Flagged***).
4	Click ***OK***.

Fitting affinity and kinetics data

See *Appendix C Principles of kinetic and affinity analysis, on page 204* for a description of the principles of fitting models to experimental data.

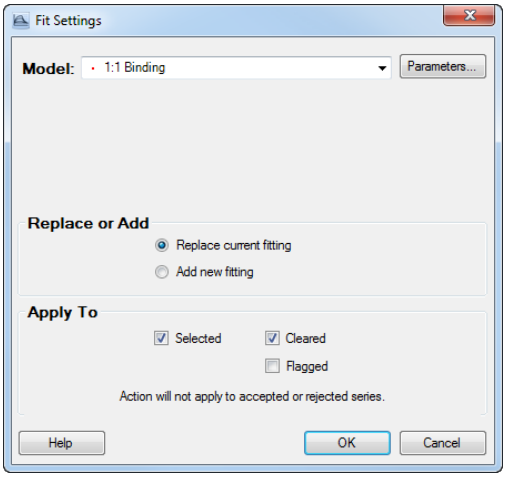
The basic fitting procedure is the same for both affinity and kinetics, although the detailed appearance differs. Follow the steps below to evaluate the data.

Step	Action
1	Choose ***Settings*** →***Fit Settings*** in the thumbnail panel.

Step	Action

Result:

The ***Fit Settings*** dialog is displayed.



2 Select the fitting model and provide parameters as required (see *Appendix D Fitting models for kinetics and affinity, on page 209*).

3 Select the series to which the model should be applied (***Selected***/***Cleared***/***Flagged***).

4 Choose whether to replace the current fitting or add a new fitting. The default selection is to replace the current fitting

5 Click ***Parameters*** if you need to edit any starting values for parameters or enter values that are missing in custom models.

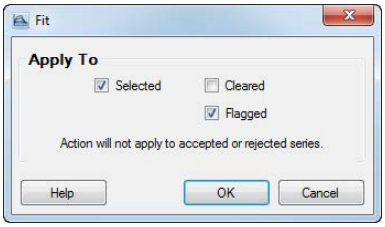
6 Click ***OK***. The choice of model is shown in the ***Results Summary*** table in the left-hand panel, and also in the tool-tip for the thumbnails and in extended thumbnails.

Note:

*The fitting is added to the detail panel when you click **OK** in the **Settings** dialog, but is not performed until you choose **Fit**.*

7 If you want to apply different models to different series in one fitting operation, repeat the steps above with different models and choice of series.

8 Click ***Fit*** in the thumbnail panel.

Step	Action
	Result: The ***Fit*** dialog is displayed. 
9	Select the series to which the fitting procedure should be applied (***Selected***/***Cleared***/***Flagged***).
10	Click ***OK***. ***Note:*** *The fitting will fail for a series if required parameters have not been provided in the* ***Fit Settings*** *dialog. A warning is issued if fitting has failed for one or more series.*
11	The fitting procedure can take some time, particularly for kinetic evaluation. Click ***Cancel*** in the progress dialog if you want to abort fitting for series that have not started.
12	If multiple fittings have been created for the same data series, choose the fitting to display at the top of the detail panel. The thumbnail panel will show the same fitting as a thumbnail.

Results summary table

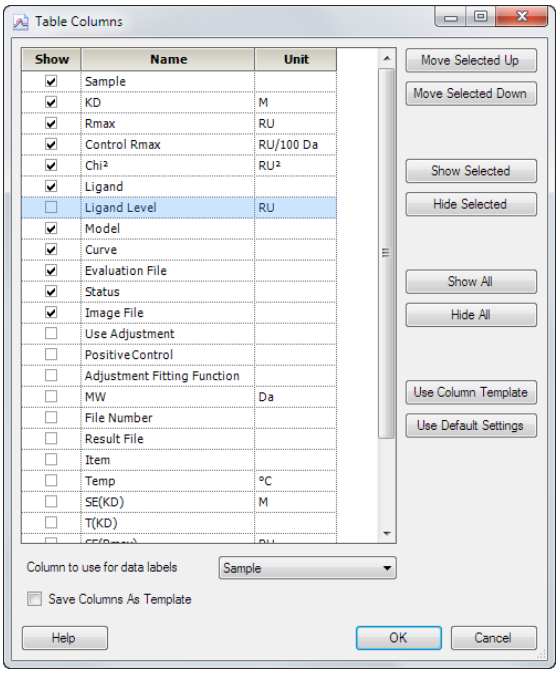
Before fitting, the table on the ***Results Summary*** tab lists details of the data series, including the model selected for each series. After fitting, the table lists the fitting results for each series.

Select a row in the table to display the details for the series in the right-hand panel. Right click on a row and choose ***Evaluation details*** to show the details in a separate window, and to set the status for the series.

Click on a column header in the table to sort the table by the content of that column. Click repeatedly to toggle the sort order.

Table columns

Click ***Table Columns*** at the top of the ***Results Summary*** panel to select which columns should be included in the summary table.



Check ***Save Columns As Template*** to save the table column settings as a template on the local computer. A saved template will be used as default when a new result file is opened. If this box is not checked, the settings will only be saved with the evaluation file and will not be applied to evaluation of other result files. Separate templates are saved for kinetic screen, affinity screen and kinetics summary. Click ***Use Column Template*** to apply the saved template to the current evaluation. Click ***Use Default Settings*** to restore the column selection to that originally provided with the software.

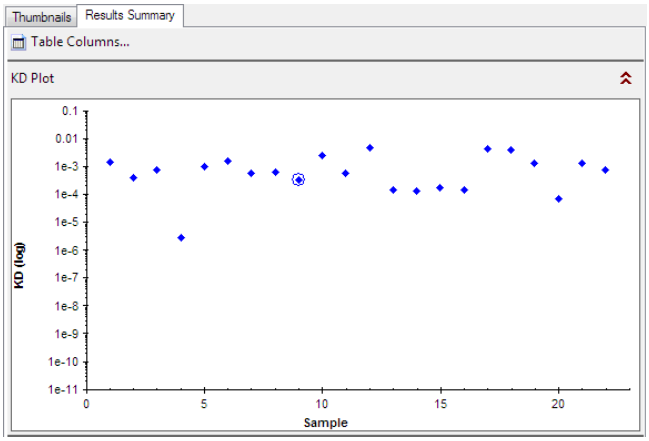
Results summary plots

The top panel in the ***Results Summary*** shows a graphical presentation of the results. The presentation differs for affinity and kinetics evaluations. In both cases, right click in the plot and choose ***Show data labels*** to label the points in the plot with the sample name.

KD plot

The summary plot for affinity evaluation is a KD plot, showing calculated affinity constants (K_D) with samples distributed on the x-axis.

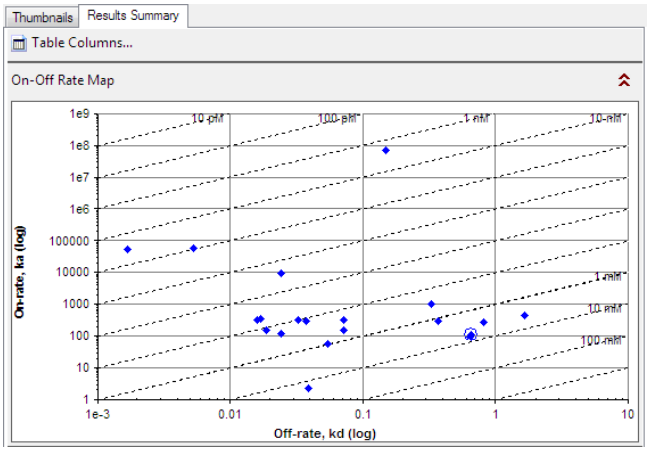
Click on a point in the plot to highlight the corresponding row in the table. Right-click for options to show the evaluation details in a separate window and to set the status for the series.



On-off rate map

The summary plot for kinetics evaluation is an on-off rate map, providing an overview of kinetic and affinity properties by plotting the association rate constant k_a against the dissociation rate constant k_d, both on logarithmic scales. Since the affinity constant K_D is the ratio of k_d to k_a, interactions that have the same affinity will appear on diagonal lines representing the K_D value. The diagonals are shown as broken lines on the plot with the K_D value indicated. Points that are separated on the same diagonal represent interactions with the same affinity but different kinetics.

Click on a point in the plot to highlight the corresponding row in the table. Right-click for options to show the evaluation details in a separate window and to set the status for the series.



11.4 Dealing with low affinity interactions

Introduction

Simple steady state affinity evaluation is generally not satisfactory for low affinity (high K_D) interactions, since the analyte concentration series should extend above the K_D value for reliable fitting, and it is sometmes not practicable or even possible to use sufficiently high concentrations. The issue is further complicated in work with many low molecular weight analytes, and in particular fragments, which exhibit binding to a specific site together with very weak binding to multiple sites on the ligand.

The difficulty of determining low affinities arises from the uncertainty in fitting a value for the maximum binding capacity R_{max} when there is insufficient curvature in the plot of R_{eq} against C. Setting a fixed value for R_{max} stabilizes the fitting procedure and allows more reliable determination of steady state affinity. Models are provided in the software for single- and multi-site affinity using constant R_{max} (see *Steady state affinity with constant Rmax, on page 218*). The value for constant R_{max} is obtained from separate measurements on a positive control, either by injecting a high concentration to apporach R_{max} or fitting to kinetic or affinity measurements, The R_{max} for a given analyte is calculated from the control value by adjusting for the molecular weight:

$$Rmax_{analyte} = Rmax_{control} \times \frac{MW_{analyte}}{MW_{control}}$$

Note: *Fitting with a constant R_{max} requires that molecular weight is specified for the analyte, in order to adapt the control R_{max} value to the individual analyte.*

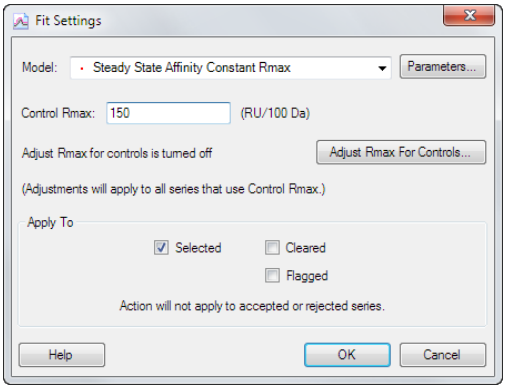
*The constant R_{max} functionality described in this section can be used with custom models provided that they are defined with a parameter **Rmax** that is set to **Constant** with initial value blank (see Appendix Models for steady state affinity, on page 227).*

Using constant R_{max}

Follow the steps below to perform fitting with constant R_{max}.

Step	Action
1	Choose ***Settings*** →***Fit Settings***.

Step	Action



2 Select a model for constant R_{max}. The predefined models provided with the software are ***Steady State Affinity Constant Rmax*** or ***Steady State Affinity Constant Rmax (Multi Site)*** [1]

3 Enter a value for ***Control Rmax***. This is the molecular weight adjusted R_{max} for the positive control in RU/100 Da.

Click ***Adjust Rmax for Controls*** if required. This will adjust the R_{max} value for drift in the control response during the assay (see below).

4 Select the series to which the model should be applied (***Selected***/***Cleared***/***Flagged***).

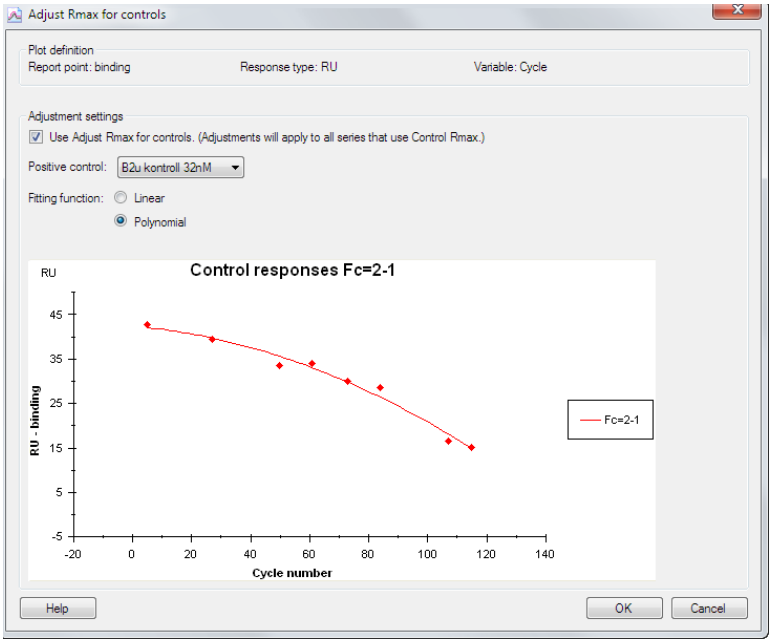
5 Click ***OK***.

1 The model for multi-site affinity with constant R_{max} handles substances that show binding to more than one site. For correct results it is important that the positive control substance binds only to one site. The constant R_{max} term applies to the site defined by the positive control binding.

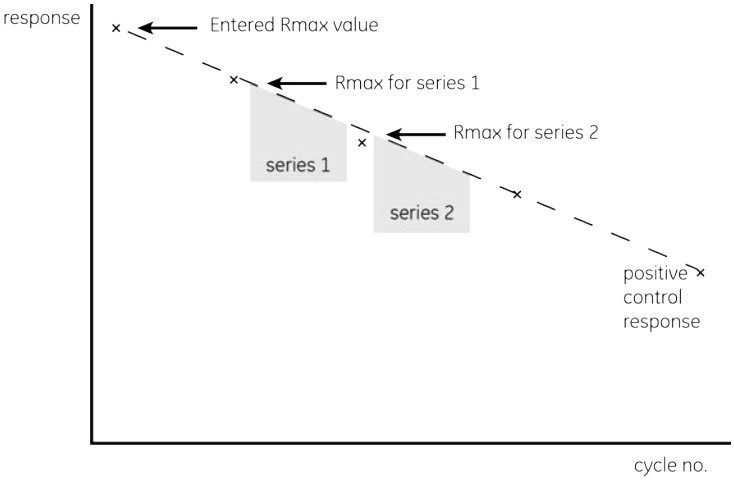
Adjusting R_{max} for controls

The value for the constant R_{max} can be adjusted automatically to compensate for changes in surface activity during the course of the run. Follow the steps below to do this.

Step	Action
1	Click ***Adjust Rmax for Controls*** in the ***Fit Settings*** dialog.
2	Check ***Use Adjust Rmax for controls***.
3	Select the positive control on which the adjustment should be based (this does not have to be the same substance as that used to determine the constant R_{max} value).
4	Choose either linear or polynomial fitting (see *Adjustment for controls, on page 125*).



5	Click ***OK***.

Step	Action
	Result: The R_{max} value for each data series will be adjusted based on the first cycle number in the series (see illustration below). The R_{max} value remains constant within the series. 

11.5 Annotations and comments

Annotating results

Data series for kinetic and affinity screen can be annotated using the ***Annotations*** function as described for result plots in *Section 9.3 Annotations and comments, on page 120*. The list of available annotations is common to result plots and kinetic/affinity evaluation items.

Note: *Annotations cannot be applied to single data points or sensorgrams in screening items.*

Include annotation and comment columns in the result table if you want annotations to be included in data export and printing.

11.6 Exporting kinetics and affinity results

Export functions

Results from kinetics and affinity evaluation can be exported one by one as curves or tables using the general export functions in graphs and tables (see *Appendix A.1 Exporting data, on page 194*). In addition, you can right-click in the thumbnail panel of a completed evaluation and choose ***Export All Graphs and Tables*** to create a folder based on the same name as the evaluation file, containing images all thumbnails as displayed in .png format and the results summary table in tab-separated text format. Only columns currently shown in the results summary are exported, with the addition of a column for the exported thumbnail image file name. Each time the ***Export All Graphs and Tables*** operation is performed on the same evaluation session, a new folder is created, distinguished in the folder name by numbers representing the number of data series in the folder.

11.7 Kinetic summary

Introduction

You can summarize the kinetics and affinity evaluation results from several saved evaluation files or from several evaluation items in the same file using the separate Biacore S200 Kinetics Summary software (installed automatically together with the Evaluation Software). Kinetic summary offers summary and presentation functions similar to those in the left-hand panel of kinetic and affinity evaluations, but can be applied to multiple items from the same or different files.

Kinetic summaries can include both affinity and kinetic evaluations in the same summary.

Creating a kinetic summary

Follow the steps below to create a kinetic summary:

Step	Action
1	Open the ***Biacore S200 Kinetic Summary*** software (separate from the Biacore S200 Evaluation Software). The software can be started from Windows or from the ***Tools*** menu in the Biacore S200 Evaluation Software.
2	Open one or more result files containing kinetics or affinity evaluation items. Use the ***File*** →***Append*** option to add files to an existing summary. You can also open or append saved kinetic summary files (file extension ***.bks***).

Summary presentation

Multiple fits in the same item in the Evaluation Software are presented as separate fits in the summary. Summary data is presented on four tabs:

Tab	Description
Table	Presents a table of the summarized kinetics and affinity data.
Thumbnail	Presents thumbnails of the kinetic and affinity evaluations included in the summary.
On-off rate map	Presents an overview of kinetic and affinity properties for the interactions in the summary as an on-off rate map.
Steady-state KD plot	Presents a plot of steady state affinity constants against sample.

Table

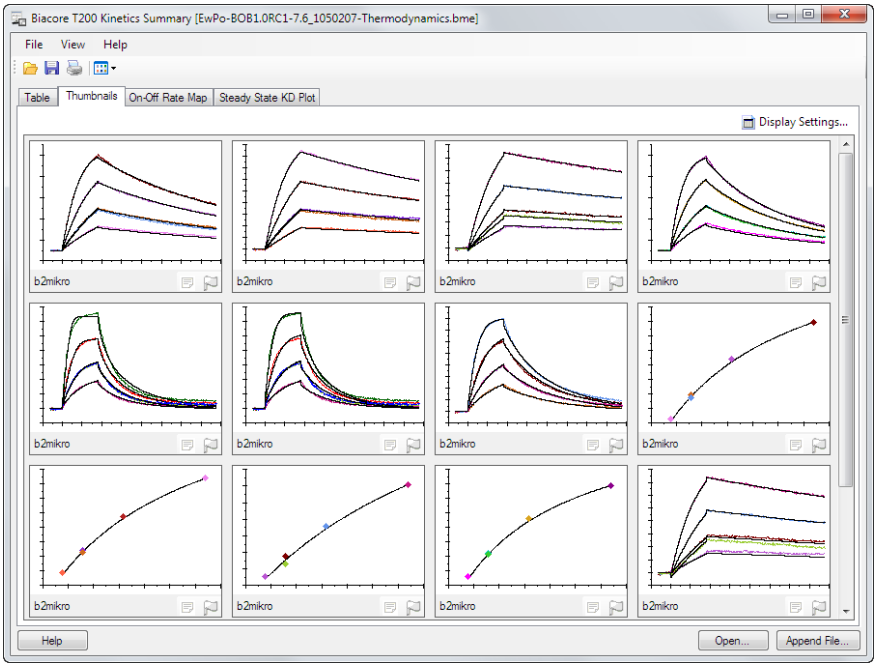
The ***Table*** tab shows a table of the summarized kinetics and affinity data. Remove the checkmark in the ***Show*** column to hide samples in the other tabs.

Click on a column header to sort the table by the contents of the column. Sorting the table also sorts the thumbnails on the ***Thumbnails*** tab.

Use the ***Table Columns*** button to choose which columns to include in the table.

Double-click on a row in the table or choose ***Evaluation details*** from the right-click menu to display the evaluation details in a separate window.

Thumbnail



See *Thumbnails, on page 144* for thumbnail presentation options.

On-off rate map

Choose the ***On-Off Rate Map*** tab to show the on-off rate map (see *On-off rate map on page 157*) for the kinetics summary. The plot does not include steady state affinity items, or kinetic evaluation items with multiple sets of kinetic constants, such as evaluation with heterogeneous models.

Click on a point in the on-off rate map or select a row in the table below the map to display the thumbnail for the evaluation.

Steady-state KD plot

Choose the ***SteadyState KD Plot*** tab to show the equilibrium dissociation constant K_D plotted against sample for steady state affinity data (see *KD plot on page 156*). Affinity constants obtained as the ratio of rate constants are not included. Duplicate sample series are plotted as separate samples.

Copying and exporting summaries

Use the right-click menu on the ***Thumbnail*** tab to copy or export kinetic summary content. See *Appendix A.1 Exporting data, on page 194* for details of the copy and export options.

12 Assessing kinetics and affinity results

About this chapter

This chapter describes how to assess the quality and reliability of kinetics and affinity evaluations.

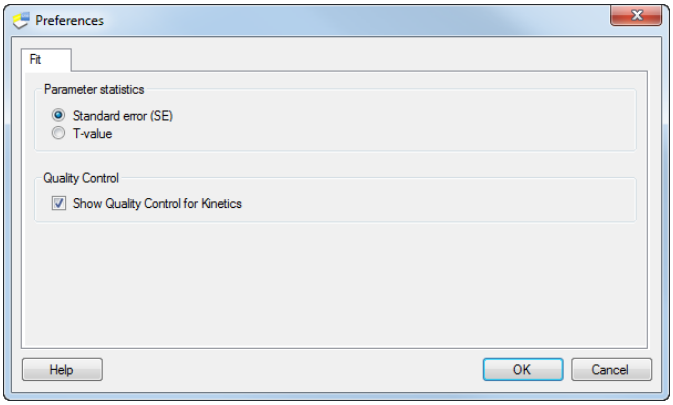
In this chapter

Section		See page
12.1	Software preferences	168
12.2	Statistical parameters	169
12.3	Quality control tab	171
12.4	Other tools for kinetics assessment	175
12.5	Assessing affinity evaluation	178

12.1 Software preferences

Settings

Choose ***Tools*** →***Preferences*** from the main menu to set preferences for kinetics and affinity evaluation.



Option	Description
Parameter statistics	Choose whether to show statistics for fitted parameters as SE- or T-values (see *SE or T-value, on page 169*).
Quality control	Choose whether to show or hide the ***Quality Control*** tab for kinetic evaluation (see *Section 12.3 Quality control tab, on page 171*).

12.2 Statistical parameters

Introduction

This section describes the statistical parameters calculated by the software to aid in assessing the results.

Chi-square

Chi-square is a measure of the average squared residual (the difference between the experimental data and the fitted curve), and is an indicator of how closely the fitted curves agree with the experimental data. A lower chi-square indicates closer fitting. One chi-square value is reported for the whole fitting.

Chi-square is calculated as

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

Parameter	Description
r_f	Fitted value at a given point
r_x	Experimental value at the same point
n	Number of data points
p	number of fitted parameters

For sensorgram data used in kinetic fitting, the number of data points is very much larger than the number of fitted parameters in the model, so ***(n-p)*** approximates to ***n***, and the chi-square value approximates to the average squared residual per data point. This approximation does not hold for affinity data, where the number of data points is the same order of magnitude as the number of fitted parameters.

Chi-square is listed on the ***Report*** tab.

SE or T-value

SE (standard error) or T-value is an indicator of parameter significance, and is reported separately for each fitted parameter on the ***Parameters*** tab.

The standard error represents an estimate of how much variation in the fitted parameter value is required to affect the closeness of fit by a given amount. Lower SE values indicate higher significance.

The T-value is obtained by dividing the value of the parameter by SE, and may be easier to interpret for comparison between parameters with widely different absolute values (e.g. k_a and k_d). A high T-value corresponds to a low SE.

The choice of whether to display parameter significance as standard error or T-value is made on the ***Fit*** tab of the ***Tools*** →***Preferences*** dialog.

As a general guideline, fitted parameters with SE greater than about 10% of the parameter value (a T-value less than about 10) have low significance for the fitting. If the SE value is higher than the parameter value (T-value less than 1), the parameter cannot be reliably determined from the experimental data. Typically (but not always), parameters with a low significance have unreasonable values: for example typical values for the mass transfer constant for proteins are around 10^8 RU·$M^{-1}s^{-1}$, but evaluation of data with no mass transfer limitation might return a value of 10^{12} or higher.

Note: *Even if parameters with low significance can have a wide range of values without affecting the fit, repeated evaluation of the same data set will always return the same value. Consistency of a value between repeated evaluations is* ***not*** *a test of significance.*

U-value

In some situations, it may be possible to determine a value for two or more parameters in combination without being able to determine unique values for the individual parameters. Such parameters are said to be correlated. One example is the kinetic rate constants k_a and k_d, that are correlated through the affinity constant K_D ($K_D = k_d/k_a$). It may sometimes be possible to determine the affinity constant reliably without being able to resolve the individual rate constants. The U-value is an estimate of the correlation between the calculated values for rate constants and R_{max}.

The U-value is determined by testing the dependence of the fit on correlated variations in pairs of parameters, and is listed on the ***Report*** tab as a single value for the whole fitting. U-values above about 25 indicate that absolute values for two or more of the parameters (rate constants and R_{max}) are correlated and cannot be determined uniquely. The value does not give any indication of which pair(s) of parameters are correlated. If the U-value is below about 15 the parameter values are not significantly correlated.

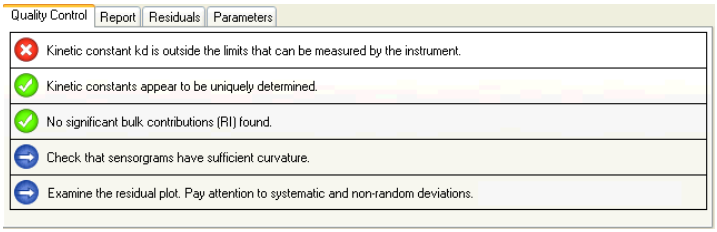
The U-value is calculated for the predefined 1:1 kinetic model only.

12.3 Quality control tab

Introduction

For evaluations that use the predefined 1:1 kinetic model, the ***Quality Control*** tab gives a brief overview of selected quality aspects. If you prefer, you can hide the quality control tab by setting the appropriate option in ***Tools*** →***Preferences*** on the main menu.

Note: *The **Quality Control** tab can only be displayed for evaluations that use the predefined 1:1 kinetics model.*



The symbols used on this tab have the following meanings:

Symbol	Color	Meaning
	Green	Pass: quality assessment acceptable.
	Yellow	Warning: quality assessment close to the limits of acceptability
	Red	Fail: quality assessment unacceptable
	Blue	User assessment recommendations

The quality control assessment covers five aspects:

- Magnitude of kinetic constants
- Parameter uniqueness
- Bulk refractive index
- Sensorgram curvature
- Residuals

NOTICE

Use the ***Quality Control*** tab as a help in making your own judgement of the results. ***Pass*** status in the quality control parameters does not necessarily indicate that the fit is acceptable or that the results are biologically relevant. On the other hand, ***Fail*** status in any of the parameters is a reliable warning indicator.

Base your assessment on the overall quality of the results and the fitting, taking all quality control parameters into account.

Magnitude of kinetic constants

If either association or dissociation rate constants are close to or outside the limits that can be determined in the instrument, this will be reported. For values close to the limit, judge the validity of the results on other assessment criteria as described in this chapter.

Parameter uniqueness

The parameter uniqueness assessment corresponds to the U-value (see *U-value, on page 170*).

Note: *This test does not explore all possible parameter correlations. A* ***Pass*** *status for this test is not a fail-safe indication that parameters are uniquely determined.*

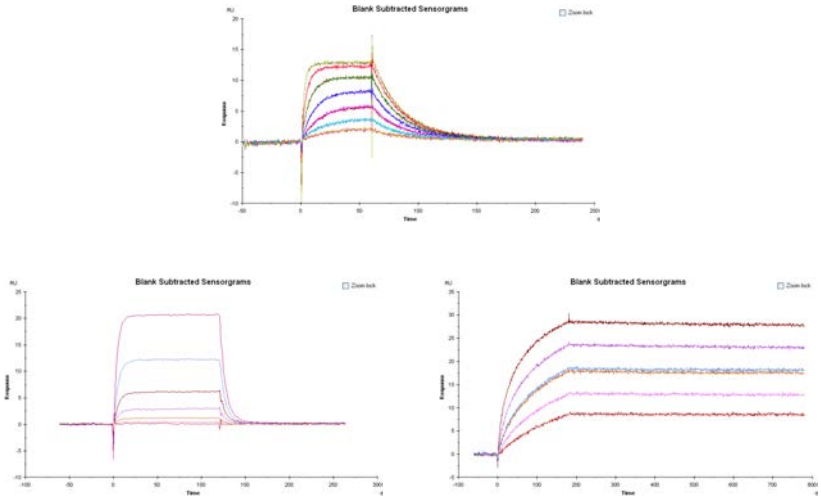
Bulk refractive index

After reference subtraction and blank subtraction, sensorgrams for kinetic evaluation should not in principle contain any bulk refractive index shifts (parameter ***RI*** in predefined models). However, there may be some circumstances where small bulk refractive index shifts may remain in reference- and blank-subtracted data. On the other hand, the fitting algorithm tends to interpret rapid interaction events (incorrectly) as bulk shifts. If the fitting returns significant values for ***RI***, a warning will be issued in the ***Quality Control*** tab.

Examine the sensorgrams and fitted curves to determine whether bulk shifts as reported by the fitting are true or false. If you think that the reported bulk shifts may actually reflect rapid binding events, you may want to set ***RI*** to a constant value of zero in the ***Parameters*** setting for the fitting.

Sensorgram curvature

You should check that the sensorgrams have sufficient curvature for kinetic determination. Ideally, the sensorgrams for at least the one or two highest concentrations should show measurable binding rates at the beginning of the sample injection and approach a steady state towards the end of the injection. Sensorgrams that approximate to "square-wave" pulses (indicating rapid association and dissociation) and those that do not flatten out during the injection generally do not contain sufficient kinetic information for reliable evaluation. Ideally, the dissociation phase should be long enough to monitor a fall in response of at least 10% to 15% of the starting value.



Examples of sufficient and insufficient sensorgram curvature.

Top: Ideal sensorgrams approaching steady state during sample injection and returning to baseline during dissociation.

Bottom left: Rapid interaction approaching "square wave" appearance. These sensorgrams return rate constants close to the limit of measurement for the instrument.

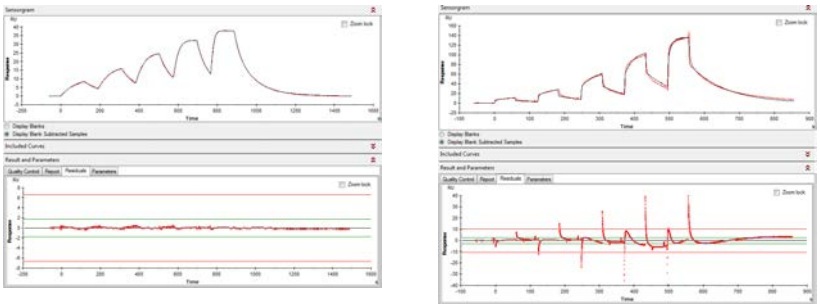
Bottom right: Slow association and dissociation, giving insufficient curvature in both association and dissociation phases. Evaluation is possible but will not be very reliable.

If the interaction is too fast to provide kinetic information, you may only be able to determine affinity constants. Interactions that do not flatten out sufficiently during the injection or dissociate sufficiently during the dissociation phase may sometimes be analyzed by prolonging the association or dissociation phase respectively.

This item is always reported as a user assessment recommendation.

Residuals

You should check that the residuals (the difference between experimental and fitted value for each data point in the sensorgrams) lie within reasonable limits. For a perfect fit, the residuals reflect the short-term noise in the sensorgrams and scatter around zero (typically ±1 to 2 RU). Systematic deviations, seen as a definite shape in the residual plot, indicate that the interaction model is to a greater or lesser extent unsuitable for the interaction.



The residuals for a good fit (left) scatter around 0, ideally in a random distribution representing the noise in the sensorgrams. For a poor fit (right) the residual curves show a definite shape and deviate farther from 0.

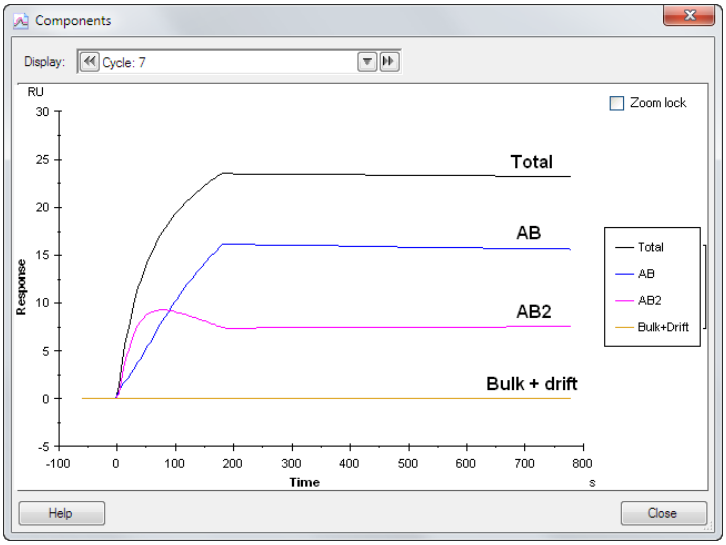
As an aid in judging the residuals, guidelines are drawn on the residual plot to indicate the range of acceptability. Most of the residuals should be within the inner (green) limits. The guideline positions are calculated in relation to the response range of the sensorgrams. The guidelines are only shown for evaluations using the predefined 1:1 kinetics model (i.e. when the quality control tab is included).

This item is always reported as a user assessment recommendation.

12.4 Other tools for kinetics assessment

Components of the fit

Choose ***Tools*** →***Components*** from the detail panel to display a plot showing the contribution of components in the interaction model to the fitted curve. Choose which cycle to display in the selector bar.

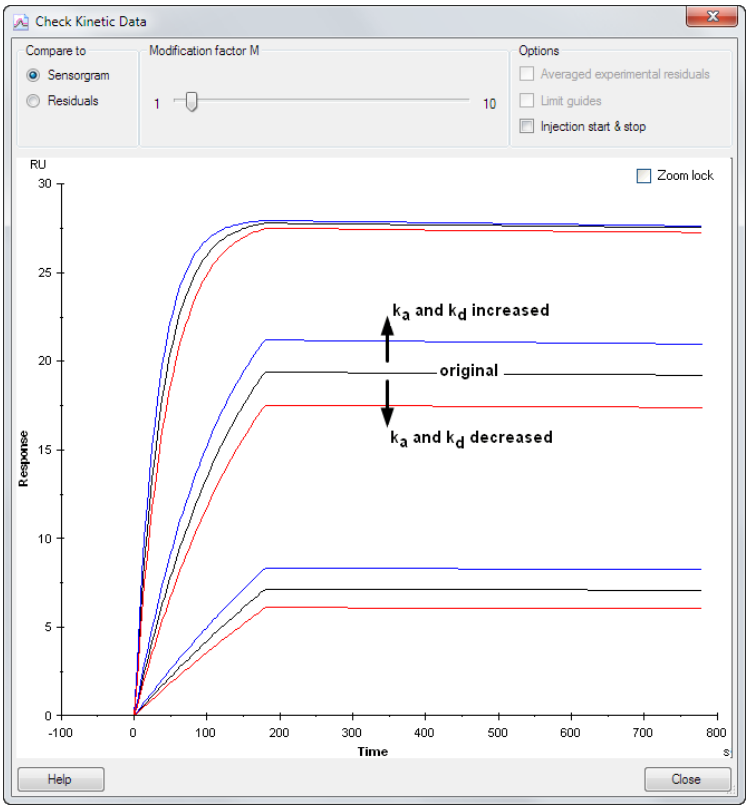


The example illustrated here is taken from a fitting to a bivalent analyte model (see *Bivalent analyte kinetics, on page 212*), and shows clearly how the component ***AB2*** (analyte attached to the surface through both binding sites) is displaced by ***AB*** as the interaction progresses.

Kinetic data check

Choose ***Tools*** →***Check Kinetic Data*** to open a window that displays simulated sensorgrams based on the fitting results, with the interaction rate constants k_a and k_d varied in parallel so that the affinity constant K_D remains unchanged. If curves do not shift as values for k_a and k_d are changed, this means that the actual values are not important for the fitting and the curves do not contain kinetic information, although the affinity constant may be correct. Conversely, if the simulated curve shape changes as the values of k_a and k_d are varied, the fitting is dependent on the actual values and the curves do contain kinetic information.

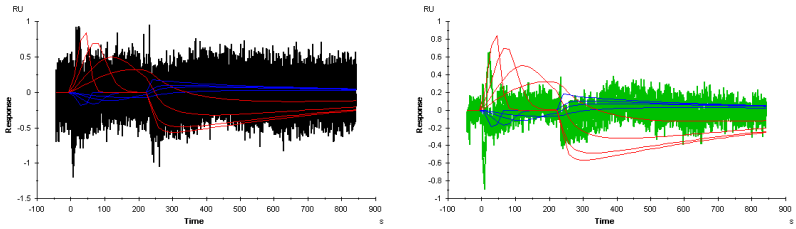
In effect, this tool tests whether the observed binding is limited by mass transport. Significant kinetic data is only obtained when mass transport is not limiting: under mass transport-limited conditions, kinetics cannot be uniquely determined but affinity constants may be reliable.



(This example is illustrated with only three concentrations for clarity.)

To use the tool, drag the slider for the modification factor ***M*** and observe the behavior of the curve display. The original curves (which remain unchanged as you drag the slider) are shown in black: blue curves show the simulation for k_a and k_d multiplied by ***M***, while red curves show the simulation for k_a and k_d divided by ***M***. If the red and blue curves clearly diverge from the original curves, the fitting is sensitive to changes in the rate constants and the curves probably contain significant kinetic information. If on the other hand the divergence is negligible, the values of the rate constants do not matter and the binding is limited by mass transport. Mass transport limitation places an upper limit on the rate constants that can be measured: on the borderline, the fitting is sensitive to a reduction in rate constants but not to an increase.

Choose the ***Residuals*** option in the ***Compare to*** frame to show the residuals for the simulated curves in relation to the experimental residuals. Choose ***Averaged experimental residuals*** in the ***Options*** frame to smooth the experimental residuals by averaging over a moving time window (the simulated residuals are not affected). Choose ***Limit guides*** to display movable horizontal lines marking the extent of residual variation, to aid visual interpretation. (Note that the limit guides are not related to the guidelines shown on the residual tab for QC purposes (see *Residuals, on page 174*), and do not in any way imply acceptance limits.)



Kinetic data check comparison to residuals (left) and averaged residuals (right).

The ***Check Kinetic Data*** tool is only available for results obtained with the predefiend 1:1 kinetics fitting model.

Note: *Standard error, kinetic data check and U-value are all tests of the significance of the fitted parameters. Standard error tests the significance of single parameters. Kinetic data check specifically examines the correlation of k_a and k_d, and is directly related to the issue of mass transport limitation. The U-value is a single value derived from the more complex correlation matrix of k_a, k_d and R_{max}.*

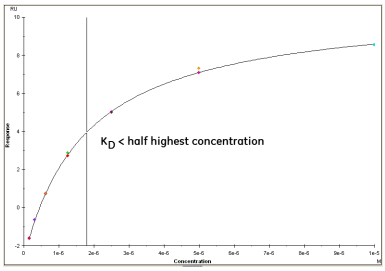
12.5 Assessing affinity evaluation

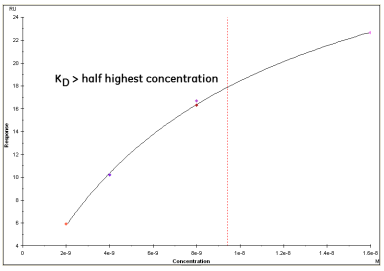
Considerations

Steady state affinity evaluations are performed by fitting a plot of R_{eq} against concentration C to a model representing equilibrium 1:1 binding. The closeness of fit is reported as a chi-square value, calculated in the same way as for kinetics. Note however that the number of points in the steady state affinity plot is very much lower than for kinetic evaluation, so that chi-square is a more sensitive indicator of fitting quality.

The plot of R_{eq} against C approaches a limiting value (equivalent to R_{max}) at high concentrations. Robust evaluation of the data requires either that the plot shows sufficient curvature for reliable estimation of R_{max} or that a valid constant value is provided for R_{max}. As a rule of thumb, evaluation with fitted R_{max} is acceptable only if the calculated K_D value is less than half the highest analyte concentration used. (For a 1:1 interaction, the K_D value is equal to the analyte concentration that gives 50% saturation of the binding sites, so that $R_{eq} = 0.5 \cdot R_{max}$. In other words, reliable evaluation is only obtained if the surface is more than 50% saturated at the highest analyte concentration.)

To help in this assessment, the calculated K_D value is indicated as a vertical line at the corresponding analyte concentration. The line is red and broken if the value is greater than half the highest concentration.





For weak interactions where it is not possible to use concentrations approaching $0.5 \cdot R_{max}$, evaluation using a model with constant R_{max} (see *Section 11.4 Dealing with low affinity interactions, on page 158*) may give more reliable results. This approach requires that R_{max} is determined separately, for example using high concentrations of a known binder.

13 Affinity in solution

About this chapter

Determination of affinity in solution provides an alternative to steady state affinity measurements for interactions that take a long time to reach equilibrium or for any other reason are difficult to determine with a direct binding assay. In principle, the affinity in solution approach uses Biacore S200 to determine the free concentration of one interactant in equilibrium mixtures containing known total interactant concentrations.

This chapter describes evaluation of affinity in solution.

In this chapter

Section		See page
13.1	Conventions and background	180
13.2	Evaluation of affinity in solution	182

13.1 Conventions and background

Experimental setup

The interactants in affinity in solution determination are denoted A and B:

$$A + B = AB$$

Experiments are set up so that a fixed concentration of B is mixed with variable concentrations of A and allowed to reach equilibrium. The free concentration of B is then determined by injecting the sample over a ligand that binds B but not A or the complex AB (the interactant A or a derivative thereof is usually suitable as ligand). It is assumed that the measurement itself does not significantly disturb the equilibrium in the sample.

The experimental setup requires a calibration curve with known concentrations of B determined over the same sensor surface, in order to calculate the free B concentrations in the samples.

Evaluation principles

The equilibrium constant for a 1:1 interaction is given by

$$K_D = \frac{A_{free} \cdot B_{free}}{AB}$$

or equivalently

$$K_D = \frac{(A_{tot} - AB)(B_{tot} - AB)}{AB}$$

Rearranging this equation gives

$$K_D \cdot AB = A_{tot} \cdot B_{tot} - AB(A_{tot} + B_{tot}) + AB^2$$

or

$$AB^2 - AB(A_{tot} + B_{tot} + K_D) + A_{tot} \cdot B_{tot} = 0$$

Substituting in the relationship $B_{free} = B_{tot} - AB$ gives

$$B_{free} = \frac{(B_{tot} - A_{tot} - K_D)}{2} \pm \sqrt{\frac{(A_{tot} + B_{tot} + K_D)^2}{4} - A_{tot} \cdot B_{tot}}$$

This equation can be fitted to a plot of B_{free} against A_{tot} to calculate a value for K_D. (Formally, the equation has two solutions, but one is always negative and is not meaningful in the context of an affinity determination.)

Requirements

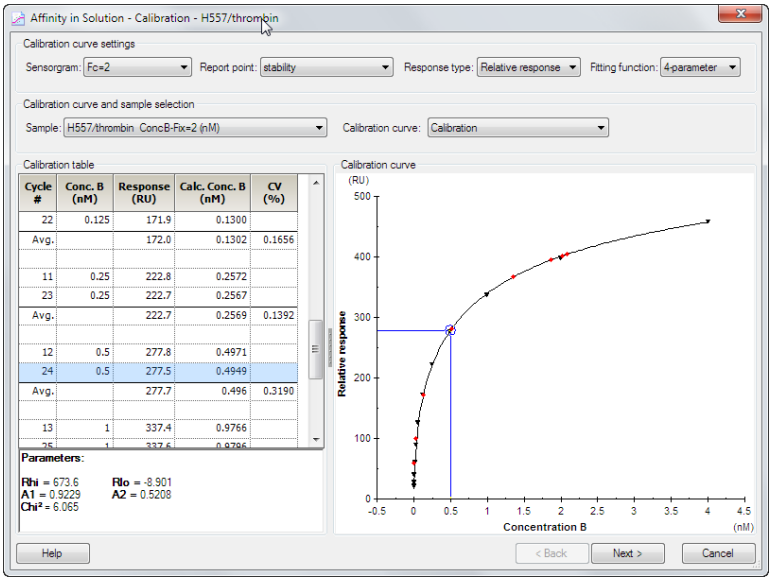
Requirements for evaluation of affinity in solution are listed below.

- At least one assay step is required with purpose ***Calibration*** and one with purpose ***Sample***. Both assay steps must be connected to a cycle type that includes one ***Sample*** injection. The two assay steps will normally be connected to the same cycle type.
- Samples in the ***Calibration*** step must have concentrations specified in the variable ***ConcB-calibration***. At least two different concentrations are required for linear calibration curves and at least four for 4-parameter fitting. These samples should contain only component B.
- Samples in the ***Sample*** step must have concentrations specified in the variables ***ConcB-fix*** and ***ConcA-variable***. At least 3 samples with the same concentration of component B mixed with different concentrations of component A are required.

13.2 Evaluation of affinity in solution

Follow the steps below to evaluate affinity in solution measurements.

Step	Action
1	Open the result file. The file contents must meet the requirements for affinity in solution evaluation (see *Requirements, on page 181* for details).
2	Click the ***Affinity in Solution*** button in the navigator panel. *Result:* The Calibration screen opens for determining the concentration of free B in the samples.
3	Choose the sensorgram, report point, response type and fitting function at the top of the dialog.
4	If you have run multiple sample series in the experiment, choose the sample to evaluate in the ***Sample*** list. A sample series is defined as all cycles with the same sample name in the assay step(s) with purpose ***Sample***.
5	If you have run multiple calibration curves in the experiment, choose the curve to use in the ***Calibration curve*** list. A calibration curve is defined as measurements from assay step(s) with purpose ***Calibration***, regardless of the sample name. If two or more ***Calibration*** assay steps are run contiguously with no intervening steps with a different purpose, they will be combined into a single calibration curve.



Step	Action

Note:

All samples in a series are evaluated against the chosen calibration curve. You cannot use different calibration curves for different samples in the same series.

The table lists the data for the calibration curve. The plot panel shows the curve with calibration points as black inverted triangles and sample points as red squares. Samples that lie outside the range of the calibration curve are not shown. Right click on calibration points to exclude the points from the curve.

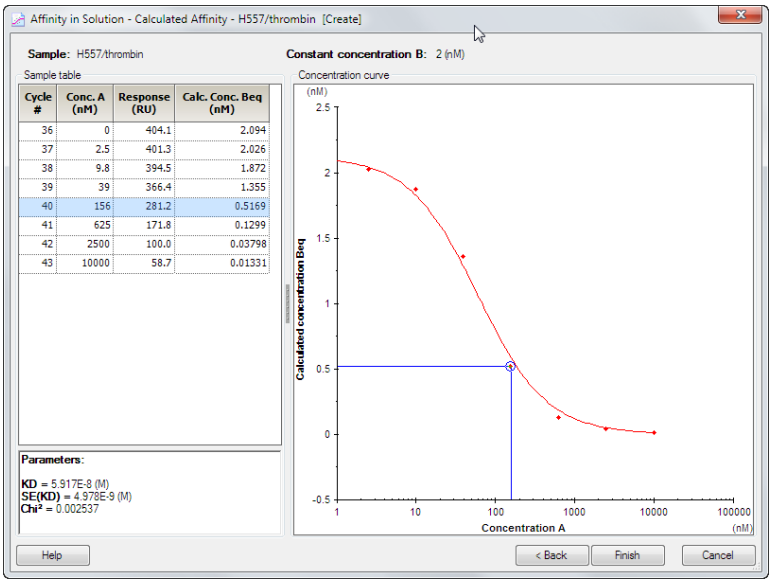
6 Click ***Next>*** to calculate the results.

Result:

The calculated concentrations of free B are displayed, plotted against the concentration of A (by default on a logarithmic scale for the concentration of A). The data are fitted to a steady state model for 1:1 binding (*Evaluation principles, on page 180*).

Note:

*Zero values cannot be plotted on a logarithmic scale. If you have included a sample with zero concentration of A in the sample series and want to display this point on the plot, choose **Scale** from the right-click menu in the plot panel and set a linear scale for the x-axis.*



Step	Action
	The table shows the numerical results for the sample series. Samples that lie outside the range of the calibration curve are marked as ***N/A*** in the column for ***Calc. Conc. Beq.***
	Note:
	The intercept of the fitted curve on the y-axis represents a fitted value for the parameter ***ConcB*** *(the total concentration of B in the samples). This value should be the same as or close to the value entered for the variable* ***ConcB*** *in the method.*
7	The calculated K_D value is shown together with fitting statistics in the panel below the table.

14 Thermodynamic analysis

About this chapter

This chapter describes the evaluation of thermodynamics using Biacore S200.

Note: *The Biacore S200 system is not appropriate for strict formal thermodynamics measurements since it is not a closed system. The analysis is based on measured temperature dependence of kinetic and affinity constants, and serves in many situations as a workable approximation to formal thermodynamics.*

In this chapter

Section		See page
14.1	Background	186
14.2	Performing thermodynamic analysis	189

14.1 Background

Scope

This section presents thermodynamic theory as it applies to the dependence of kinetic and afffinity parameters on temperature.

Equilibrium thermodynamics

For equilibrium thermodynamics, the *van't Hoff equation* states:

$$\Delta G^\circ = -RT \ln \frac{1}{K_D} = RT \ln K_D$$

where

Parameter	Description
ΔG°	Standard Gibbs free energy change
R	Universal gas constant
T	Absolute temperature (K)
K_D	Equilibrium dissociation constant

Substituting in the expression

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$$

and rearranging gives

$$\ln K_D = \frac{\Delta H^\circ}{RT} - \frac{\Delta S^\circ}{R}$$

where

Parameter	Description
ΔH°	Standard enthalpy change
ΔS°	Standard entropy change

A plot of ln K_D against 1/T should thus be a straight line, with slope $\Delta H^\circ/R$ and intercept on the y-axis ΔS°.

This simplified relationship does not hold if the heat capacities of reagents and products differ, since different amounts of energy will be required to raise the temperature by the same amount on the two sides of the reaction. In such cases, the plot of ln K_D against 1/T is not linear, and the relationship becomes

$$RT\ln K_D = \Delta H^\circ_{T_0} - T\Delta S^\circ_{T_0} + \Delta C^\circ_P(T - T_0) - T\Delta C^\circ_P \ln\left(\frac{T}{T_0}\right)$$

where

Parameter	Description
$\Delta C_P°$	Heat capacity change under standard conditions
T_0	Reference temperature (25°C = 298.15 K for standard conditions)

A value for the standard heat capacity change $\Delta C_P°$ can thus in principle be obtained in addition to $\Delta H°$ and $\Delta S°$ from non-linear fitting of the data to this extended equation.

Transition state thermodynamics

Transition state theory holds that the equilibrium constant for formation of the transition state in a reaction can be related to the rate constant for the overall reaction by the *Eyring equation*:

$$K^\ddagger = \frac{k\hbar}{k_B T}$$

where

Parameter	Description
K‡	Equilibrium constant for formation of the transition state for the forward or back reaction
k	Kinetic rate constant for the interaction in the corresponding direction (k_a or k_d)
$\hbar$	Planck's constant
k_B	Boltzmann's constant

Applying a similar rearrangement of the thermodynamic equations for the transition state gives:

$$\ln\frac{k\hbar}{k_B T} = -\frac{\Delta H^{o\ddagger}}{RT} + \frac{\Delta S^{o\ddagger}}{R}$$

so that the thermodynamic transition state constants for the forward and backward reactions can be obtained from plots of $\ln(k_a/T)$ and $\ln(k_d/T)$ respectively against 1/T.

Note that the Eyring equation does not have a corresponding non-linear form that takes account of the heat capacity change for transition state formation. Non-linear fitting to obtain values for $\Delta C_P°$ can only be applied to equilibrium thermodynamic analysis.

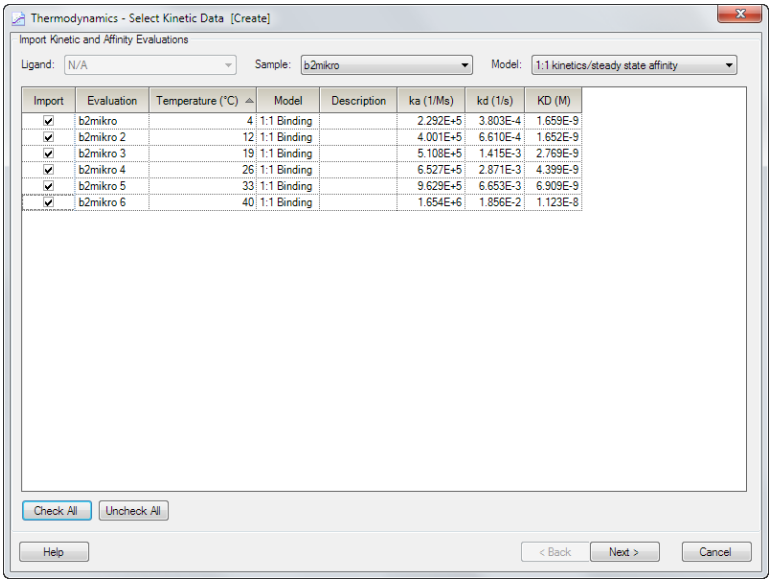
14.2 Performing thermodynamic analysis

Procedure

Thermodynamics evaluation requires that kinetics or affinity is determined at two or more temperatures (recommended 5).

Follow the steps below to perform a thermodynamic analysis.

Step	Action
1	Evaluate the kinetics and/or affinity at each temperature required for the thermodynamics analysis, using the same fitting model for each item. Create a separate ***Kinetics/Affinity*** evaluation item at each temperature.
2	Open a new ***Thermodynamics*** evaluation item. *Result:* The ***Create*** dialog opens, listing the finished kinetic and affinity evaluation items in the current session.
3	Choose the ligand, sample and fitting model for the evaluation. You may only choose one ligand-sample combination, and you should only choose one fitting model. Options for the model are ***1 → 1 kinetics/steady state affinity*** (recommended) or ***All***. Do not combine data from different fitting models in the same evaluation.
4	Check the Import box for the rows that you want to use in the evaluation. Use ***Check All*** and ***Uncheck All*** as quick options to select and deselect the whole list.



Step	Action

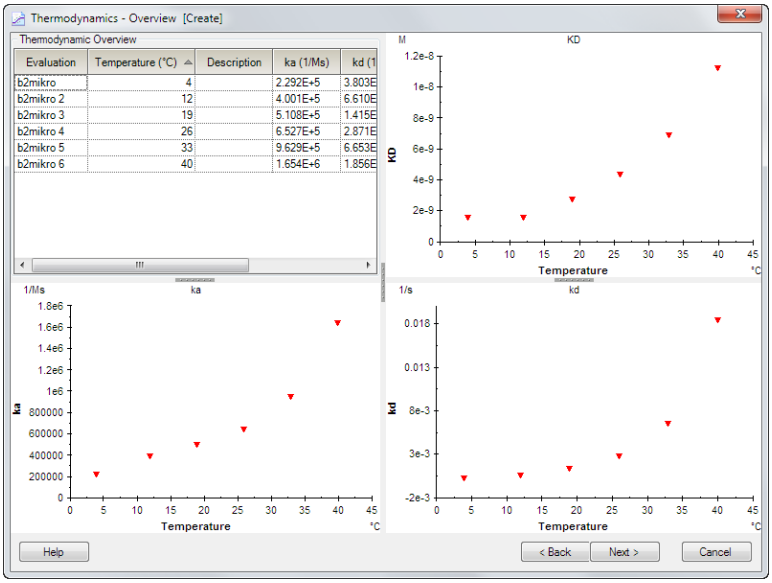
Note:

If you use data from fitting models that include multiple rate or affinity constants, be sure to select the correct rows so that equivalent constants are included from each fit. In some cases it may be necessary to examine the kinetic or affinity evaluation items to determine which constants belong together.

5 Click ***Next>***.

Result:

Plots of affinity and rate constants against temperature are displayed.

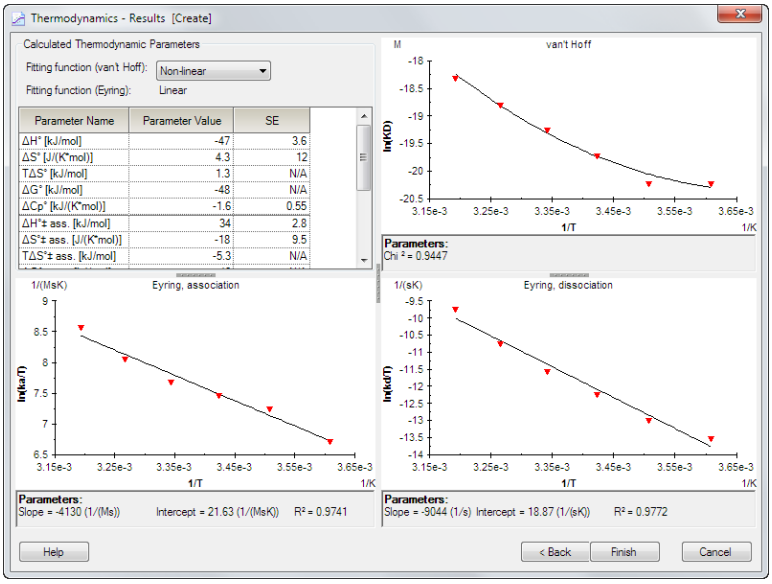


6 Click ***Next>***.

Step	Action

Result:

The results are displayed as van't Hoff and Eyring plots together with a table of thermodynamic constants for the equilibrium and transition state formation. Right-click on a point in any of the plots to exclude the point from the evaluation.



7 Choose whether to use a linear or non-linear fitting function for the van't Hoff plot (see *Equilibrium thermodynamics, on page 186*). If you choose non-linear fitting, a value for ΔC_P will be included in the reported parameters. Energies of activation (E_a), derived from the Eyring plots, are also listed for the transition states. All thermodynamic parameters are calculated for a temperature of 25°C.

Note:

Regardless of the setting for the van't Hoff plot, the Eyring plots are always fitted to a linear function. Calculation of ΔC_P by non-linear fitting is not valid for transition state data (see Transition state thermodynamics, on page 187).

If you have combined kinetic and steady state affinity data in the thermodynamic evaluation, the van't Hoff plot will show all affinity values, but the Eyring plots will be empty because the steady state data lacks values for the rate constants.

Plots of kinetic and affinity constants against temperature show temperature values in °C, while van't Hoff and Eyring plots use absolute temperature values (K).

Step	Action
8	Click ***Finish*** to finalize the thermodynamic analysis.

When assessing the validity of thermodynamic constants reported by this analysis procedure, pay particular attention to the kinetic analysis at different temperatures. With complex interactions involving macromolecules, there is a significant possibility that the characteristics of the interaction (including the extent of mass transport limitation) change with temperature, resulting in different fitting quality at different temperatures. This may be evident from direct comparison of the kinetic fits, but will not be immediately apparent in the thermodynamic analysis.

Appendix A
Data import and export

About this appendix

This appendix describes facilities for importing and exporting data to and from Biacore S200 software.

In this chapter

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A.1	Exporting data	194
A.2	Importing data	197

A.1 Exporting data

Export functions

Several options are available for exporting data from Biacore S200 software to third-party programs. General options are summarized in the table below and described in more detail in the sections that follow.

Option	Context	Destination	Comments
Right click, ***Copy Table***	Most tables containing numerical data	Windows clipboard	Copied to tab-separated text as shown on the screen
Right click, ***Copy Graph***	Most sensorgram displays, plots, and charts	Windows clipboard	Copied as a graphical object
Right click, ***Export curves***	Most sensorgram displays and plots	Windows clipboard	Data point coordinates exported as tab-separated text
File →Export to Excel ***File →Export to XML***	Control and Evaluation Software	Excel or XML file	All session content exported (see below)
File →Export Report Point Table	Control and Evaluation Software	Text file	Entire report point table exported regardless of columns, sorting and filtering

There are also some export facilities restricted to specific functions, such as export of rack positions from the Rack Positions dialog in the Control Software and export of screening results with both graphical thumbnails and numerical data from finished kinetics and affinity screening evaluation items.

Export formats

Export to Excel

To export data to an Excel file, choose ***File →Export to Excel***.

Export from the Control Software creates an Excel spreadsheet file (extension ***.xls***) containing separate worksheets for the file properties and report point table.

Export from the Evaluation software creates an Excel spreadsheet file (extension ***.xls***) containing separate worksheets for the file properties and for tabulated data for all evaluation items where appropriate (i.e. plot data and evaluation results). The worksheets for each item are identified with the item name. For plots, only the columns shown in the plot window table are exported. Data from sensorgram items is not exported.

Export to XML

To export data to an XML file, choose ***File*** →***Export to XML***.

This option exports the same data as to Excel but creates a text file in XML format (file extension ***.xml***). Details of the XML format are most easily determined by exporting data from the Control or Evaluation Software and opening the exported file in an XML-compatible editor.

The illustration below shows an example of the beginning of an XML export file.

```
<?xml version="1.0" encoding="utf-8"?>
<EvaluationFile>
  <FileProperties>
    <EvalFileProperties>
      <EvaluationFile>
      </EvaluationFile>
      <UserInformation>
        <PerformedBy>501815</PerformedBy>
        <CurrentUser>501815</CurrentUser>
      </UserInformation>
      <CreatedWithSoftware>
        <Name>Biacore T200 Evaluation Software</Name>
        <Version>3.0</Version>
      </CreatedWithSoftware>
      <Notebook><![CDATA[]]></Notebook>
    </EvalFileProperties>
    <ResultFiles>
      <ResultFile1>
        <ResultFile>
          <Name>LMW screen.blr</Name>
          <Path>C:\Users\501815\Documents\Test run</Path>
          <Size>1 215 488 bytes</Size>
        </ResultFile>
        <RunInformation>
          <Type>Method Builder</Type>
          <Method>(Untitled)</Method>
          <Cycles>24</Cycles>
          <Start>20/01/2014 10:15:20</Start>
          <End>20/01/2014 14:27:02</End>
        </RunInformation>
        <Instrument>
          <InstrumentType>BiacoreT200</InstrumentType>
          <InstrumentId>1358419</InstrumentId>
          <IFC>TYPE105</IFC>
        </Instrument>
        <UserInformation>
```

Context-specific export

Report point table

To export the report point table to a tab-separated text file, choose ***File*** →***Export*** →***Report Point Table***. The exported file has the extension .rpt. The entire report point table is exported regardless of settings for ***Table columns***, sorting, and filtering. Use ***Copy Table*** from the right-click menu to copy the report point table as displayed on the screen.

Note: *If you open an exported report point table in Microsoft Excel, make sure that the format for the **Fc** column is set to **Text** in the Excel import file wizard. The default setting of **General** for text file import may interpret the flow cell identification for reference-subtracted data as a date instead of a text string.*

Kinetics and affinity

To export both thumbnail graphs and numerical data from kinetic and affinity screening items, choose ***Export All Graphs and Table*** from the right-click menu in the thumbnail panel of the finished item. This will create a folder based on the same name as the evaluation file, containing images of all thumbnails as displayed files in ***.png*** format and the results summary table in tab-separated text format. The file name for each exported thumbnail is listed in the ***Image file*** column of the results summary. Each time the ***Export All Graphs and Tables*** operation is performed on the same evaluation session, a new folder is created, distinguished in the folder name by numbers representing the number of data series in the folder.

Rack positions

Rack positions can be exported from the Control Software to a tab-separated text file in either ASCII or Unicode format using the ***Menu*** →***Export Positions*** function in the ***Rack Positions*** dialog (see *Rack positions dialog, on page 69*). The file contains two lines identifying the microplate and reagent rack settings followed by the contents of the rack positions table with the columns separated by tabs.

A.2 Importing data

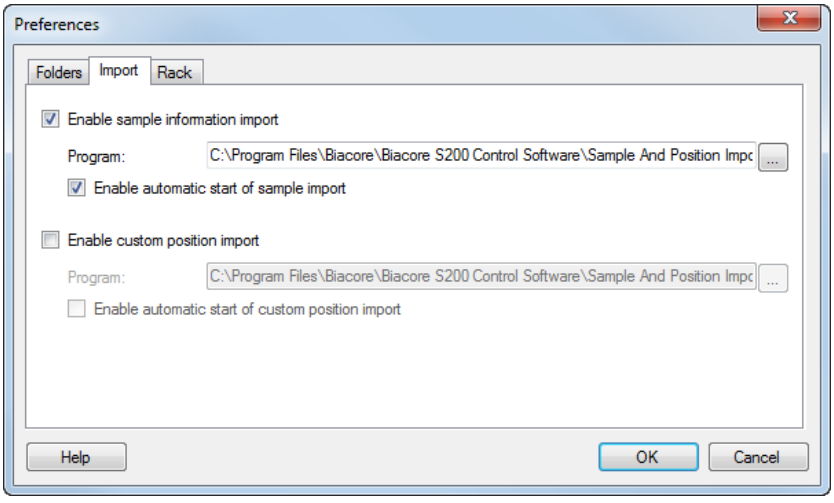
Import options

Options for importing data are summarized in the following table.

Software	Import options
Control Software	The Control Software supports import of sample data and positioning information to the sample table and ***Rack positions*** dialogs respectively (see *Section 5.8 Setup Run, on page 65*). Import options are controlled through the ***Tools Preferences*** settings.
Evaluation Software	The Evaluation Software supports import of model definitions for kinetics and affinity evaluation. Model files for import should be obtained from Cytiva or created by exporting models from another installation.

Import settings

In order to use the import functions in the Control Software, the appropriate options must be selected in ***Tools*** →***Preferences*** and valid import programs must be specified. A default ***Sample and Position Import*** program is installed with the software. See the on-line help for details of how to use this program.



For each import function, a check box allows the import program to be started automatically without user intervention. If the respective box is not checked, the program will only be started when the user actively requests data import. If the box is checked:

- Data is imported automatically to the sample table only if the table is empty. The program is not started if the table already contains data.

- Data is imported to the ***Rack Positions*** dialog whenever automatic positioning is invoked. This happens when the dialog is first opened with new or modified sample data, and also when the user requests Automatic positioning from the dialog menu (Section 4.2.6). The program is not started when the user makes manual changes to the rack positioning or when the dialog is opened with no changes in previously positioned samples (for example when the user clicks ***Back*** and ***Next*** in the dialog sequence without changing sample information).

Sample information import

When the sample import function is invoked, the contents of the sample table are first exported in Extensible Markup Language (XML) format to a temporary file that is submitted to the specified import program. The import program may append new sample data to the file or overwrite the file contents with new data as required. The modified file is then imported back into the sample table and the temporary file is deleted.

Development or choice of a suitable import program is the responsibility of the user. To document the detailed XML format of the import file, specify an XML-compatible text editor as the import program and save a copy of the import file from a suitable table.

The illustration below shows an example of the beginning of an XML sample import file.

```
<?xml version="1.0" encoding="iso8859-1" ?>
<!DOCTYPE MethodBuilderImport (View Source for full doctype...)>
- <MethodBuilderImport importFileVersion="1.0">
  - <AssaySteps>
    - <AssayStep name="Startup">
      - <DataTable row="1">
          <Data cmd="Sample 1" grp="" fld="Solution" val="sample 1/2" />
          <Data cmd="Sample 1" grp="" fld="Conc1" val="0" />
          <Data cmd="Sample 1" grp="" fld="Conc2" val="125" />
          <Data cmd="Sample 1" grp="" fld="MW1" val="200" />
          <Data cmd="Sample 1" grp="" fld="MW2" val="10000" />
        </DataTable>
      </AssayStep>
    - <AssayStep name="Sample">
      - <DataTable row="1">
          <Data cmd="Sample 1" grp="" fld="Solution" val="sample 1/2" />
          <Data cmd="Sample 1" grp="" fld="Conc1" val="0" />
          <Data cmd="Sample 1" grp="" fld="Conc2" val="0" />
          <Data cmd="Sample 1" grp="" fld="MW1" val="200" />
          <Data cmd="Sample 1" grp="" fld="MW2" val="10000" />
        </DataTable>
      - <DataTable row="2">
          <Data cmd="Sample 1" grp="" fld="Solution" val="sample 1/2" />
          <Data cmd="Sample 1" grp="" fld="Conc1" val="0" />
          <Data cmd="Sample 1" grp="" fld="Conc2" val="195" />
```

Rack positions import

The ***Menu*** →***Custom Position Import*** and ***Simple Position Import*** functions in the ***Rack Positions*** dialog (see *Rack positions dialog, on page 69*) import rack position data from an external tab-separated text file such as one from a laboratory robot used to prepare sample microplates. If you choose ***Custom Position Import***, the external file is first processed by the import program as specified in ***Files*** →***Preferences***. Output from this program must be tab-separated text in either ASCII or Unicode format conforming to the specification below. The ***Simple Position Import*** option imports data directly from a text file conforming to the specifications with no intervention from an external program.

Rack positions import file format

Format requirements for the text file are listed below.

- Two lines in the file specify the microplate and reagent rack settings, in the format

  `Rack1=<microplate specification>`

  `Rack2=<reagent rack specification>`
- Specifications are not case-sensitive, but microplate and reagent rack specifications must be given otherwise exactly as they appear in the selection lists in the ***Rack Positions*** dialog. If either specification is invalid, the corresponding definition will not be imported. The position of these two lines in the file does not matter.
- One line specifies the headers for table columns to be imported, separated by tabs. The headers should correspond to the column headers as they appear in the ***Rack Positions*** table, with the exception of the Volume column in the table which can be omitted from the import file (and is ignored if it is present). This line may not be preceded by any line other than the microplate and reagent rack specifications.
- A set of lines hold the content of the table columns separated by tabs. Each line must contain the same number of tab characters as the header line.

Details of the required import file format can be investigated further by examining a file created with the ***Menu*** →***Export Positions*** command. The illustration below shows an example file as it appears when opened in Microsoft Excel.

	A	B	C	D	E	F
1	Rack1=96 Well Microplate					
2	Rack2=Reagent Rack 2					
3	Position	Volume (µl)	Content	Type	Sample 1 Conc (µ	Sample 1 MW (Da)
4	R1 A1	88	a	Sample	1	
5	R1 A2	88	b	Sample	0	
6	R1 A3	88	b	Sample	1	
7	R1 A4	88	b	Sample	1	
8	R1 A5	88	b	Sample	2	
9	R1 A6	88	b	Sample	3	
10	R1 A7	88	b	Sample	4	
11	R1 A8	88	b	Sample	5	
12	R1 A9	88	a	Sample	0	
13	R1 A10	88	a	Sample	1	
14	R1 A11	88	a	Sample	2	
15	R1 A12	88	a	Sample	3	
16	R1 B1	88	a	Sample	4	
17	R1 B2	88	a	Sample	5	

Rack positions import process

When import is requested, the contents of each table line in the import file are matched as far as possible to the contents of the ***Rack Positions*** table, with the exception of the ***Position*** and ***Volume*** column. For matched rows, the ***Position*** in the table is replaced by the value in the ***Position*** column from the import file. Rows for which a match cannot be found are not imported. Any rows in the ***Rack positions*** table which do not have a matching row in the import file are left without a ***Position*** specification and must be placed in the microplate or reagent rack before the run can be started.

Appendix B
Organization of Biacore templates

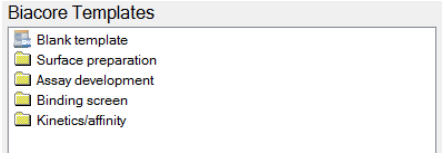
Introduction

This appendix describes the organization of the predefined Biacore templates provided with the Biacore S200 Control Software. To understand the details of how a particular template is constructed, work through the template definition as described in *Chapter 5 Methods, on page 42*.

The predefined Biacore templates are organized in four main folders on the basis of application:

- Surface preparation
- Assay development
- Binding screen
- Kinetics and affinity

A blank template is provided as a starting point for creating custom templates from scratch.

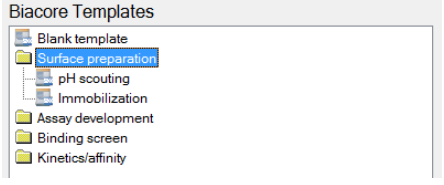


General template structure

The predefined templates are in general set up as complete methods, ready to run after completion of the sample table (see *Section 5.8 Setup Run, on page 65*). Parameters such as flow rates, injection and dissociation times and variable settings are set in accordance with recommendations from Cytiva. Settings may be changed as required.

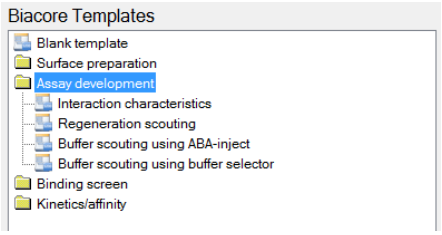
Surface preparation

The ***Surface Preparation*** folder contains templates for pH scouting and immobilization:



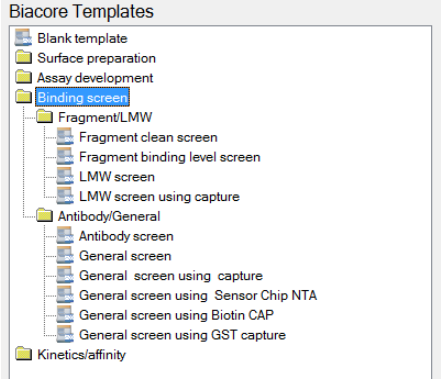
Assay development

The ***Assay development*** folder contains templates for testing interaction characteristics, regeneration scouting, and buffer scouting using either the buffer selector or the A-B-A inject (see *Sample injection, on page 59*).



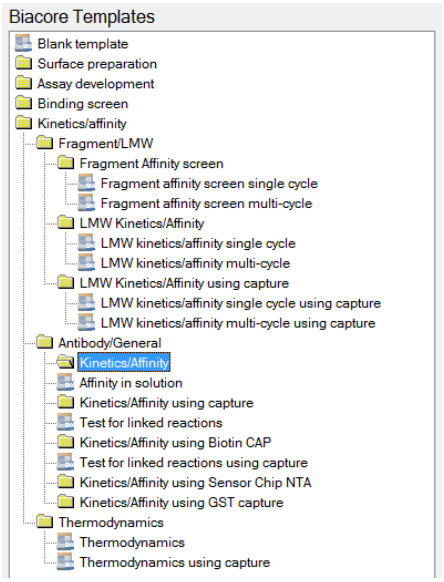
Binding screen

The ***Binding screen*** folder contains templates for screening fragments, low molecular weight (LMW) compounds and antibodies or other macromolecules for binding to the ligand. Variants are provided for captured ligand using custom capturing molecules and for capture using Sensor Chip NTA for histidine-tagged ligands, Biotin CAP for biotinylated ligands, and GST Capture Kit for GST-tagged ligands.



Kinetics and affinity

The Kinetics and affinity folder contains templates for kinetics and affinity deterrminations for fragments, low molecular weight (LMW) compounds and antibodies or other macromolecules, and for thermodynamic measurements. Variants are provided for captured ligand using custom capturing molecules and for capture using Sensor Chip NTA for histidine-tagged ligands, Biotin CAP for biotinylated ligands, and GST Capture Kit for GST-tagged ligands. Subfolders contain templates for single- and multi-cycle formats.


Biacore Templates
Blank template
Surface preparation
Assay development
Binding screen
Kinetics/affinity
Fragment/LMW
Fragment Affinity screen
Fragment affinity screen single cycle
Fragment affinity screen multi-cycle
LMW Kinetics/Affinity
LMW kinetics/affinity single cycle
LMW kinetics/affinity multi-cycle
LMW Kinetics/Affinity using capture
LMW kinetics/affinity single cycle using capture
LMW kinetics/affinity multi-cycle using capture
Antibody/General
Kinetics/Affinity
Affinity in solution
Kinetics/Affinity using capture
Test for linked reactions
Kinetics/Affinity using Biotin CAP
Test for linked reactions using capture
Kinetics/Affinity using Sensor Chip NTA
Kinetics/Affinity using GST capture
Thermodynamics
Thermodynamics
Thermodynamics using capture

Appendix C
Principles of kinetic and affinity analysis

About this appendix

This appendix describes the principles of kinetic and affinity analysis in Biacore S200.

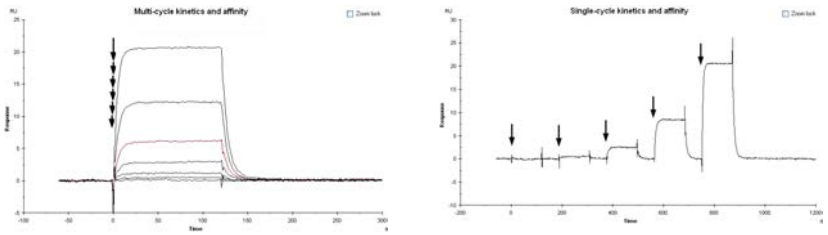
In this chapter

Section		See page
C.1	Experimental formats	205
C.2	Requirements for kinetics and affinity evaluation	206
C.3	Curve fitting principles	207

C.1 Experimental formats

Multi- and single-cycle analyses

Kinetics and affinity are normally determined from the binding characteristics of a series of analyte concentrations. These concentrations may be injected in separate cycles with surface regeneration between the cycles (multi-cycle analysis) or sequentially in a single cycle with no regeneration between injections (single-cycle analysis), as illustrated below. Results from these two approaches are evaluated in the same way, using the same tools and fitting models, and may even be evaluated together in a single evaluation.



In multi-cycle kinetics and affinity determinations (left), each sample is injected in a separate cycle. The concentration series is presented as an overlay plot aligned at the start of the injection in the evaluation software. In single-cycle determinations (right), the samples are injected sequentially in the same cycle. Arrows in the illustrations mark the start of sample injections.

C.2 Requirements for kinetics and affinity evaluation

Minimum requirements

The minimum requirements for evaluation of kinetics or affinity are one cycle with a ***Sample*** injection in an assay step with purpose ***Sample***, and with the sample concentration in the keyword ***Conc***. If the concentration is not given in molar-based units, the keyword ***MW*** must also be included with a value for the molecular weight. If necessary, the keyword table can be edited after the run is completed to meet the conditions (see *Section 7.1 Keywords, on page 85*). Note however that the injection type cannot be edited in the keyword table.

Evaluation of steady state affinity requires at least 3 non-zero analyte concentrations.

Recommendations

The recommended minimum conditions for detailed kinetic and affinity analysis are:

- a concentration series of analyte with at least three non-zero concentrations
- at least one blank cycle consisting of zero concentration sample (for single-cycle kinetics the blank cycle must replicate the sequence of injections in the analysis cycle)
- for multi-cycle kinetics, duplicate determinations for at least one non-zero concentration

Kinetic screening is often performed using fewer analyte concentrations (typically 2 or 3) and without duplicates. Affinity screening, like detailed affinity analysis, requires at least 3 (recommended 4) analyte concentrations to provide sufficient data for evaluation. Blank cycles are recommended, although the same cycle may be used as a blank for several different analytes.

C.3 Curve fitting principles

Introduction

Both kinetics and affinity are evaluated by fitting a mathematical model of the interaction to the experimental data. While a close fit between the model and the data provides some confidence in the numerical results, obtaining a good fit is not in itself evidence that the model describes the physical reality of the interaction. The fitting procedure does not have any "knowledge" of the biological significance of parameters in the model equations, and it is wise always to examine the results obtained for reasonableness of the values obtained. In addition, any mechanistic conclusions drawn for the interaction from fitting results (e.g. concerning multiple interaction sites or conformational changes) should ideally be tested using independent techniques.

Fitting procedure

Kinetic and affinity parameters are extracted from experimental data by an iterative process that finds the best fit for a set of equations describing the interaction. Equations may be created automatically from the definition of the interaction model or entered as mathematical expressions. The fitting process begins with initial values for the parameters in the equations, and optimizes the parameter values according to an algorithm that minimizes the sum of the squared residuals.

In some situations, the fitting algorithm may be unable to find a fit for the experimental data with the initial parameter values as specified in the model. This may happen typically if the concentration unit is incorrect: for example, if the unit is set to mM instead of nM in the keyword table. Occasionally, it may be necessary to adjust the starting values for fitting parameters.

Local and global parameters

Parameters in the fitting equations are treated as *local variables*, *global variables*, or *constants* as described in the following table.

Parameter type	Description
Local variables	Assigned an independent value for each curve in the data set (or sample injection in single-cycle kinetics). Typical local parameters are concentration (which is different for different curves) and bulk refractive index contribution (which may be expected to vary between curves).
Global variables	Have one single value that applies to the whole data set. Typical global parameters are the rate constants for the interaction, which should have the same value for all curves in the data set.

Parameter type	Description
Constants	Have a fixed value that is not changed in the fitting procedure. An example is the analyte concentration. Constants may also be local (separate values for each curve) or global (one value for the whole data set).

Evaluating kinetics or affinity with global rate constants gives a more robust value for the rate constants, although the curves may fit the experimental data more closely if all parameters are fitted locally. This is because local fitting allows variation between the constants obtained from different curves: when the constants are fitted globally, this variation appears in the closeness of fit rather than the reported values. Rate constants are always global in predefined kinetic models.

In general, rate constants should be fitted as global parameters and bulk refractive index contribution as a local parameter. The analyte binding capacity of the surface R_{max} is a global parameter by default in the predefined models: this assumes that the ligand activity is unchanged between cycles in the assay. It is however justified to use a local R_{max} if there is reason to believe that the ligand activity may vary between cycles (for example, in a capture assay, if the capture level varies between cycles).

The local/global parameter status is not relevant for affinity determination, since this evaluation fits the model to a single curve of response against analyte concentration.

Appendix D
Fitting models for kinetics and affinity

About this appendix

This appendix describes details of the pre-defined fitting models for kinetics and affinity, and describes how to create and edit custom models.

In this chapter

Section		See page
D.1	Predefined models	210
D.2	Creating and editing fitting models	220

D.1 Predefined models

Introduction

A set of predefined models for kinetics and steady state affinity is provided with Biacore S200 Evaluation Software. These models are marked in the model selection list with a red dot, and cannot be removed or modified. A model editor is provided for creating custom models (see *Appendix D.2 Creating and editing fitting models, on page 220*).

Mass transfer in kinetic models

All kinetic models include a term for mass transfer of analyte to the surface. If transfer is slow compared with binding of analyte to the ligand, the transport process will limit the observed binding rate, at least partially. All models take account of this potential limitation and can extract rate constants from the data provided that mass transfer is not totally limiting.

Mass transfer processes are incorporated into the interaction scheme as potentially rate-limiting transfer of analyte between bulk solution and the surface where the interaction takes place. As an example, the simple 1:1 interaction scheme may be represented as

$$A_{bulk} \underset{k_m}{\overset{k_m}{\rightleftharpoons}} A_{surface} + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$

For simplification in the model descriptions that follow, the mass transfer step is not shown in the interaction scheme although it is included in the fitting model.

Note: *Even for the simplest 1:1 interaction model, inclusion of mass transfer results in rate equations that cannot be mathematically integrated to provide a simple representation of a sensorgram (plot of response against time). For this reason, all kinetic evaluation employs numerical integration algorithms.*

Mass transfer parameters

All kinetic models include a term for mass transfer of analyte to the surface. If transfer is slow compared with binding of analyte to the ligand, the transport process will limit the observed binding rate, at least partially. All models take account of this potential limitation and can extract rate constants from the data provided that mass transfer is not totally limiting.

The rate of mass transfer of analyte to the surface under the conditions of non-turbulent laminar flow that prevail in the Biacore flow cell is characterized by the *mass transfer coefficient* k_m (units $m \cdot s^{-1}$):

$$k_m = 0.98\left(\frac{D^2 \cdot f}{0.3 \cdot h^2 \cdot w \cdot l}\right)^{1/3}$$

Parameter	Description
D	Diffusion coefficient of the analyte ($m^2 \cdot s^{-1}$)
f	Volume flow rate of solution through the flow cell ($m^3 \cdot s^{-1}$)
h, w, l	Flow cell dimensions (height, width, length in m)

One form used in fitting models in Biacore S200 is referred to as the mass transfer constant k_t (units $RU \cdot M^{-1} \cdot s^{-1}$), obtained by adjusting the mass transfer coefficient approximately for the molecular weight of the analyte and for the conversion of surface concentration to RU:

$$k_t = k_m \times MW \times G$$

where G is the conversion factor from surface concentration to RU. The value of G is approximately 10^9 for proteins on Sensor Chip CM5.

A further modification of this expression gives the flow rate-independent component of the mass transfer constant (units $RU \cdot M^{-1}s^{-2/3}m^{-1}$), referred to as t_c in the models:

$$t_c = \frac{k_t}{\sqrt[3]{f}}$$

1:1 binding kinetics

This is the simplest model for kinetic evaluation, and is recommended as default unless there is good experimental reason to choose a different model. The model describes a 1:1 interaction at the surface:

$$A + B = AB$$

Model parameters		Source
ka	Association rate constant ($M^{-1}s^{-1}$)	Fitted
kd	Dissociation rate constant (s^{-1})	Fitted
Rmax	Analyte binding capacity of the surface (RU)	Fitted
Conc	Analyte concentration (M)	Input
tc	Flow rate-independent component of the mass transfer constant	Fitted
f	Flow rate (µl/min)	Input
tOn	Sample injection start time (s)	Input
tOff	Sample injection end time (s)	Input

Model parameters		Source
RI	Bulk refractive index contribution in the sample	Fitted

Reported parameters		Source
ka	Association rate constant ($M^{-1}s^{-1}$)	ka
kd	Dissociation rate constant (s^{-1})	kd
KD	Equilibrium dissociation constant (M)	kd/ka
Rmax	Analyte binding capacity of the surface (RU)	Rmax
Conc	Analyte concentration (M)	Conc
tc	Flow rate-independent component of the mass transfer constant	tc
f	Flow rate (µl/min)	f
kt	Mass transfer constant	$tc \cdot f^{1/3}$
RI	Bulk refractive index contribution in the sample	RI

Bivalent analyte kinetics

This model describes the binding of a bivalent analyte to immobilized ligand, where one analyte molecule can bind to one or two ligand molecules. The two analyte sites are assumed to be equivalent. The model may be relevant to studies among others with signaling molecules binding to immobilized cell surface receptors (where dimerization of the receptor is common) and to studies using intact antibodies binding to immobilized antigen. As a result of binding of one analyte molecule to two ligand sites, the overall binding is strengthened compared with 1:1 binding. This effect is often referred to as avidity.

A + B = AB

AB + B = ABB

Note: *Once analyte is attached to the ligand through binding at the first site, interaction at the second site does not contribute to the SPR response. For this reason, the association rate constant for the second interaction is reported in units of $RU^{-1}s^{-1}$, and can only be obtained in $M^{-1}s^{-1}$ if a reliable conversion factor between RU and M is available. For the same reason, a value for the overall affinity or avidity constant is not reported.*

Model parameters		Source
ka1	Association rate constant for the first site ($M^{-1}s^{-1}$)	Fitted

Model parameters		Source
ka2	Association rate constant for the second site ($RU^{-1}s^{-1}$)	Fitted
kd1 kd2	Dissociation rate constant for the first and second sites (s^{-1})	Fitted
Rmax	Analyte binding capacity of the surface (RU)	Fitted
Conc	Analyte concentration (M)	Input
tc	Flow rate-independent component of the mass transfer constant	Fitted
f	Flow rate (µl/min)	Input
tOn	Sample injection start time (s)	Input
tOff	Sample injection end time (s)	Input
RI	Bulk refractive index contribution in the sample	Fitted

Reported parameters		Source
ka1	Association rate constant for the first site ($M^{-1}s^{-1}$)	ka1
ka2	Association rate constant for the second site ($RU^{-1}s^{-1}$)	ka2
kd1 kd2	Dissociation rate constant for the first and second sites (s^{-1})	kd1 kd2
Rmax	Analyte binding capacity of the surface (RU)	Rmax
Conc	Analyte concentration (M)	Conc
tc	Flow rate-independent component of the mass transfer constant	tc
f	Flow rate (µl/min)	f
RI	Bulk refractive index contribution in the sample	RI

Heterogeneous analyte kinetics

This model is intended for analysis of the kinetics of interaction of mixtures of two analytes that compete for the same ligand site. Experiments of this kind can be used to deduce kinetic parameters for a low molecular weight analyte that gives a small response from measurements of binding of a competing high molecular weight analyte. Response contributions from both analytes are taken into account, although the high molecular weight analyte is responsible for the dominant component in the observed sensorgrams.

Concentrations and molecular weights are required for both analytes. If absolute molecular weights are not known, relative values can be entered without affecting the outcome of the fitting. The model cannot evaluate interactions where the proportions and relative sizes of the analytes are unknown.:

A1 + B = A1B
A2 + B = A2B

Model parameters		Source
ka1 ka2	Association rate constant for the first and second analytes ($M^{-1}s^{-1}$)	Fitted
kd1 kd2	Dissociation rate constant for the first and second analytes (s^{-1})	Fitted
Rmax1 Rmax2	Analyte binding capacity of the surface for the first and second analytes independently (RU)	Fitted
Conc1 Conc2	Concentration of the first and second analytes (M)	Input
mw1 mw2	(Relative) molecular weights of the first and second analytes	Input
tc1 tc2	Flow rate-independent component of the mass transfer constant for the first and second analytes	Fitted
rcf	Response correction factor, allowing for different refractive index contributions for the two analytes. Default value 1.	Fitted
f	Flow rate (µl/min)	Input
tOn	Sample injection start time (s)	Input
tOff	Sample injection end time (s)	Input
RI	Bulk refractive index contribution in the sample	Fitted

Reported parameters		Source
ka1 ka2	Association rate constant for the first and second analytes ($M^{-1}s^{-1}$)	ka1 ka2
kd1 kd2	Dissociation rate constant for the first and second analytes (s^{-1})	kd1 kd2

Reported parameters		Source
KD1 KD2	Equilibrium dissociation constant for the first and second analytes (M)	kd1/ka1 kd2/ka2
Rmax1	Analyte binding capacity of the surface for the first analyte (RU)	Rmax2·rcf
Rmax2	Analyte binding capacity of the surface for the first analyte (RU)	Rmax2
Conc1 Conc2	Concentration of the first and second analytes (M)	Conc1 Conc2
tc1 tc2	Flow rate-independent component of the mass transfer constant for the first and second analytes	tc1 tc2
kt1 kt2	Mass transfer constants for the first and second analytes	$tc1 \cdot f^{1/3}$ $tc2 \cdot f^{1/3}$
f	Flow rate (µl/min)	f
RI	Bulk refractive index contribution in the sample	RI

Heterogeneous ligand kinetics

This model describes an interaction between one analyte and two independent ligands. The binding curve obtained is simply the sum of the two independent reactions. Unlike the case of heterogeneous analyte, the relative amounts of the two ligands does not have to be known in advance.

$$A + B1 = AB1$$
$$A + B2 = AB2$$

Note: *The model is limited to two ligands because the fitting algorithm tends to become unstable with more components, and three or more ligand species cannot be reliably resolved.*

Model parameters		Source
ka1 ka2	Association rate constant for the first and second ligands ($M^{-1}s^{-1}$)	Fitted
kd1 kd2	Dissociation rate constant for the first and second ligands (s^{-1})	Fitted
Rmax1 Rmax2	Analyte binding capacity of the first and second ligands (RU)	Fitted

Model parameters		Source
Conc1 Conc2	Analyte concentration (M)	Input
tc	Flow rate-independent component of the mass transfer constant	Fitted
f	Flow rate (µl/min)	Input
tOn	Sample injection start time (s)	Input
tOff	Sample injection end time (s)	Input
RI	Bulk refractive index contribution in the sample	Fitted

Reported parameters		Source
ka1 ka2	Association rate constant for the first and second analytes ($M^{-1}s^{-1}$)	ka1 ka2
kd1 kd2	Dissociation rate constant for the first and second analytes (s^{-1})	kd1 kd2
KD1 KD2	Equilibrium dissociation constant for the first and second analytes (M)	kd1/ka1 kd2/ka2
Rmax1 Rmax2	Analyte binding capacity of the first and second ligand (RU)	Rmax2·rcf
Conc	Analyte concentration (M)	Conc
tc	Flow rate-independent component of the mass transfer constant	tc
kt1 kt2	Mass transfer constant	$tc \cdot f^{1/3}$
f	Flow rate (µl/min)	f
RI	Bulk refractive index contribution in the sample	RI

Two state reaction kinetics

This model describes a 1:1 binding of analyte to immobilized ligand followed by a conformational or other change that stabilizes the complex. To keep the model simple, it is assumed that the changed complex can only dissociate through reversing the conformational change:

$$A + B = AB = AB^*$$

Note: *Conformational changes in ligand or complex do not normally give a response in Biacore systems. A good fit of experimental data to the two-state model should be taken as an indication that conformational properties should be investigated using other techniques (e.g. spectroscopy or NMR), rather than direct evidence that a conformational change is taking place.*

Model parameters		Source
ka1	Association rate constant for analyte binding ($M^{-1}s^{-1}$)	Fitted
kd1	Dissociation rate constant for the ligand-analyte complex (s^{-1})	Fitted
ka2	Forward rate constant for the stabilizing change (s^{-1})	Fitted
kd2	Reverse rate constant for the stabilizing change (s^{-1})	Fitted
Rmax	Analyte binding capacity of the surface (RU)	Fitted
Conc	Analyte concentration (M)	Input
tc	Flow rate-independent component of the mass transfer constant	Fitted
f	Flow rate (µl/min)	Input
tOn	Sample injection start time (s)	Input
tOff	Sample injection end time (s)	Input
RI	Bulk refractive index contribution in the sample	Fitted

Reported parameters		Source
ka1	Association rate constant for analyte binding ($M^{-1}s^{-1}$)	ka1
kd1	Dissociation rate constant for the ligand-analyte complex (s^{-1})	kd1
ka2	Forward rate constant for the stabilizing change (s^{-1})	ka2
kd2	Reverse rate constant for the stabilizing change (s^{-1})	kd2
KD	Overall equilibrium dissociation constant (M)	kd1/ka1 (kd2/ (ka2+kd2))
Rmax	Analyte binding capacity of the surface (RU)	Rmax
Conc	Analyte concentration (M)	Conc
tc	Flow rate-independent component of the mass transfer constant for the first and second analytes	tc

Reported parameters		Source
kt1 kt2	Mass transfer constants for the first and second analytes	$tc \cdot f^{1/3}$
f	Flow rate (µl/min)	f
RI	Bulk refractive index contribution in the sample	RI

Steady state affinity

This model calculates the equilibrium dissociation constant K_D for a 1:1 interaction from a plot of steady state binding levels (R_{eq}) against analyte concentration (C). The equation includes an offset term which represents the response at zero analyte concentration.

$$R_{eq} = \frac{CR_{max}}{K_D + C} + offset$$

Paameters		Source
KD	Equilibrium dissociation constant (M)	Fitted
Rmax	Analyte binding capacity of the surface (RU)	Fitted
offset	Residual response at zero concentration (RU)	Fitted

Note: *Reported K_D values that are higher than half the highest analyte concentration used should be treated with caution. If the response against concentration plot does not flatten out sufficiently because the concentrations are not high enough in relation to the K_D value, the reported value may be unreliable. The reported K_D value is marked as a vertical line on the fitting plot (see Details, on page 146).*

Steady state affinity with constant Rmax

This model uses the same equation as the simple steady state affinity model, but sets the R_{max} parameter to a constant. The value for R_{max} is obtained for each analyte from a value entered for a control analyte and the relative molecular weights of control and sample (see *Using constant Rmax, on page 158*). The value may be adjusted for assay drift using repeated control samples (see *Adjusting Rmax for controls, on page 160*).

$$Rmax_{analyte} = Rmax_{control} \times \frac{MW_{analyte}}{MW_{control}}$$

Multi-site steady state affinity with constant Rmax

This model fits data from interactions that exhibit binding to multiple sites. Two sites are accommodated in the model.

The model uses a constant R_{max} value for one site, defining the expected stoichiometry, and a fitted value for the other site, which can give an apparent value with undefined stoichiometry.

$$R_{eq} = \frac{CR_{max1}}{K_{D1} + C} + \frac{CR_{max2}}{K_{D2} + C} + \text{offset}$$

Parameters		Source
KD1	Equilibrium dissociation constant (M) for the high affinity site	Fitted
Rmax1	Analyte binding capacity (RU) corresponding to the defined stoichiometry	Input
KD2	Equilibrium dissociation constant (M) representing the low affinity site(s)	Fitted
Rmax2	Analyte binding capacity (RU) corresponding to the stoichiometry from free fitting	Fitted
C	Analyte concentration (M)	Input
offset	Residual response at zero concentration (RU)	Fitted

D.2 Creating and editing fitting models

In this section

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Introduction

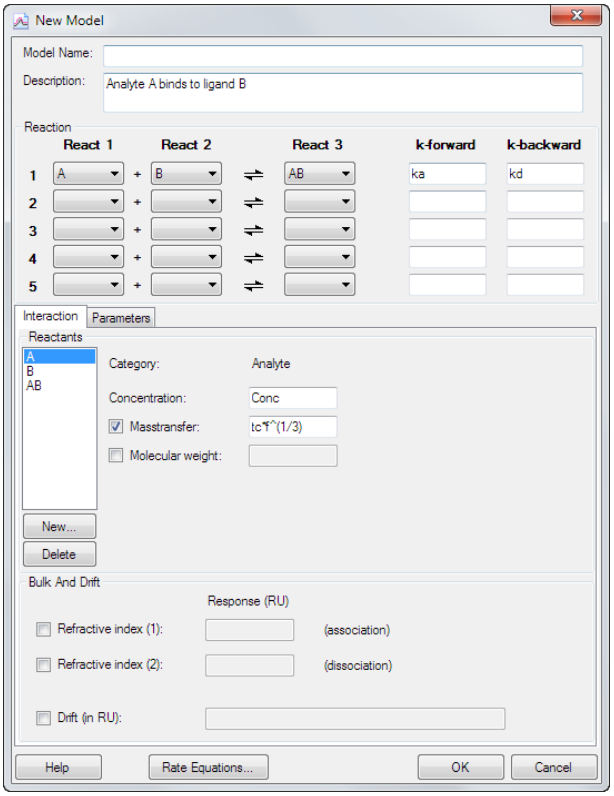
To create your own models for kinetics or affinity evaluation, choose ***Tools*** →***Models*** from the main menu and select the type of model you want to work with. You can use existing models as templates. Choose an existing model from the list and click ***New***: answer ***Yes*** in the following dialog to create a new model based on the chosen template or ***No*** to create a blank model. For kinetic models, you can define a new model either as a reaction scheme describing the interaction or as an equation defining response as a function of time.

Predefined models cannot be edited or removed. If you want to modify a predefined model, create a new model using the predefined model as a template.

Interaction models for kinetics

Introduction

Interaction models are defined as a reaction scheme with appropriate parameters. The reaction scheme supports up to five component reactions.



Tip: *Examining how predefined models are set up can help in understanding how to define interaction models.*

Model definition

Follow the steps below to define a new model or edit an existing definition.

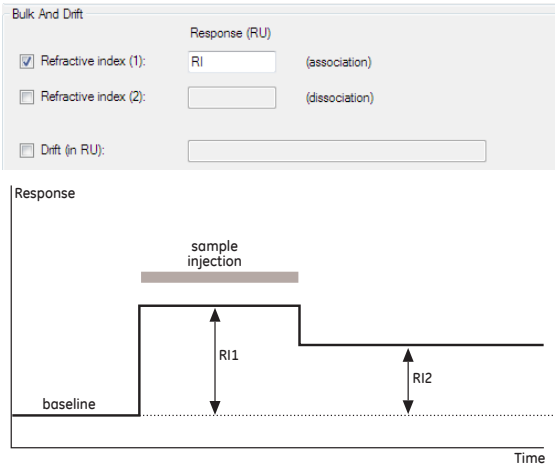
Step	Action
1	On the ***Interaction*** tab, click ***New*** to add new reactants. For each reactant, choose whether it is analyte, ligand or complex (see below) and enter an identifier for the reactant. Enter parameter names or expressions for the reactant properties.

Step	Action

Note:

Numbers are used as part of the identifier, not in the conventional chemical sense of stoichiometry. Thus a complex named AB2 contains one molecule of A and one of B2, not two molecules of B.

2 In the ***Bulk and Drift*** panel, enter details for bulk refractive index contribution. Normally, there will be one bulk refractive index term applicable during association (from the start to the end of the injection). A second term can be used if necessary during dissociation (after the end of the injection), for example to accommodate a permanent shift in baseline as a result of the sample injection.

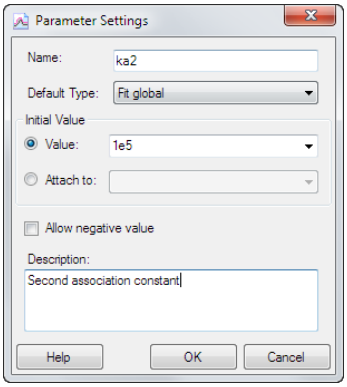


Check ***Drift*** and enter an expression describing the drift (most commonly a linear function of time) to account for baseline drift.

3 Enter the reaction scheme in the ***Reaction*** panel using the pull-down list for each reactant. Enter parameter names for the forward and backward rate constants for each line in the reaction scheme. (The terms ***k-forward*** and ***k-backward*** apply to the reaction as entered in the scheme, reading from left to right). You can also enter mathematical expressions or constant values for the rate constants.

4 Click the ***Parameters*** tab and define the parameters used in the reaction scheme. Click ***Add*** to add a new parameter, and define the parameter properties in the ***Parameter settings*** dialog.

Step **Action**



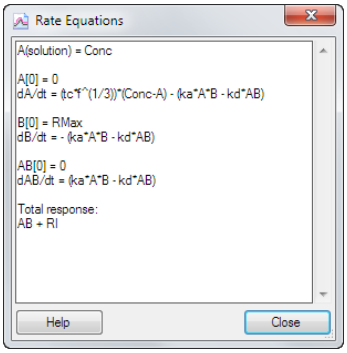
If you have only used single parameter names (as opposed to expressions) for the rate constants and properties, you can click ***Rate equations*** or ***OK*** as a shortcut to defining parameters. The software will then set up entries for all undefined parameters. This shortcut cannot be used if you have entered expressions.

- Enter the parameter name. This must correspond to the identifier used in the reaction scheme. Names are not case-sensitive.
- Choose a default type for the parameter (***Fit global***, ***Fit loca***l or ***Constant***).
- For the ***Initial value***, enter a numerical value or select a value expression from the pull-down list. The expressions represent functions evaluated within the current data set (e.g., ***Ymax*** is the maximum y-value in the data set). Alternatively, choose ***Attach to*** and select a parameter from the list. If you attach a parameter to ***Keyword***, the initial parameter value will be set to the value of the keyword with the same name as the parameter.
- Check ***Allow negative value*** if the parameter can be below zero.
- Enter a description of the parameter for ease of identification if required.

5 In the ***Report*** panel, define the parameters you want to appear in the ***Report*** tab of the results. Report parameters are defined by a name that may be chosen freely and a value that is entered as a parameter or expression containing parameters.

6 Click ***Rate Equations*** to display the equations generated by the software.

Step	Action



Select equations in the display and click ***Copy*** to copy the equations as text to the Windows clipboard.

Reactant categories

Reactants are either analyte, ligand or complex. Properties differ between the categories.

Analyte

The analyte is injected in solution at a constant concentration. Analyte is usually denoted by the letter A.

Property	Descripotion
Concentration	Injected concentration in molar units.
Mass transfer	Check this option to include a mass transfer term in the fitting model, and enter a parameter name or expression for the mass transfer constant.
Molecular weight	Check this option and enter a molecular weight if required. This information is used to calculate relative response contributions for heterogeneous analyte models.

Ligand

The ligand is immobilized or captured on the surface. Ligand is usually denoted by the letter B.

Property	Descripotion
Binding capacity	Maximum analyte binding capacity of the surface in RU.
At molecular weight	This parameters is only used in models for heterogeneous analyte. Check the option and enter the molecular weight parameter for the analyte to which the binding capacity parameter refers. Binding capacity for the other analyte will be calculated using the molecular weight values.

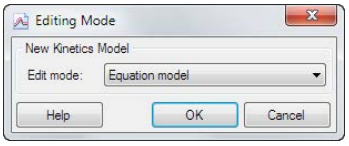
Complex

The complex is formed on the surface and normally generates response.

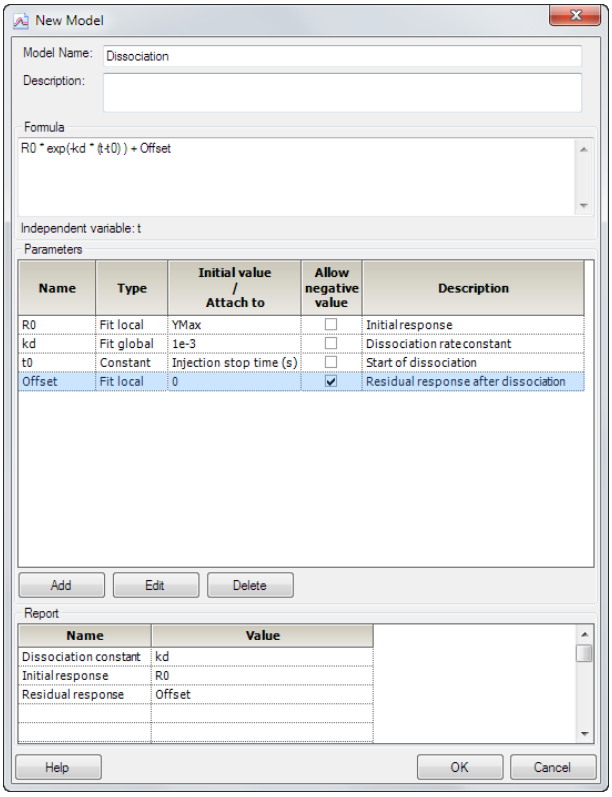
Property	Descripotion
Generates response	Uncheck this option for complexes that do not contribute to the response.
Molecular weight	Check this option and specify a parameter for complexes that form in solution and then bind to the surface. Do not check this option if ***Generates response*** is also checked.

Equation models for kinetics

Models for kinetic evaluation can also be entered as an expression defining response as a function of time t. To create an equation model, choose ***New*** in the kinetics models dialog, then choose to create the new model without using the currently selected model as a template. Select ***Equation model*** in the subsequent dialog. Parameters and report parameters are defined in the same way as for interaction models.



The example below shows a model for evaluation of the dissociation phase only.

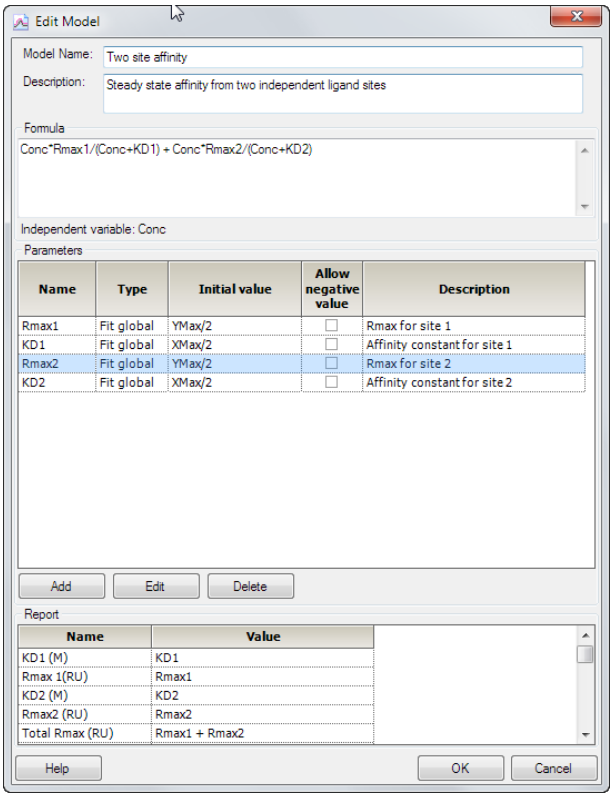


Models for steady state affinity

Models for steady state affinity evaluation are entered as an expression defining ***Req*** as a function of concentration ***Conc***. Parameters and report parameters are defined in the same way as for kinetic models.

To enable constant R_{max} functionality (see *Section 11.4 Dealing with low affinity interactions, on page 158*) in a custom model, set the ***Rmax*** parameter to a constant and leave the initial value blank.

The example below shows a model for two-site affinity evaluation.

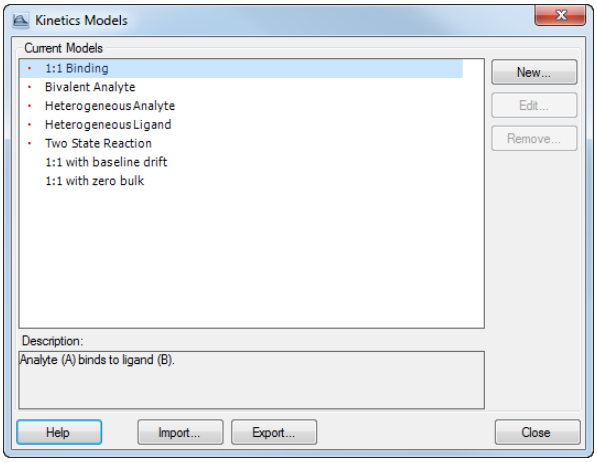


Note: *Beware of trying to define and use complex models for steady state affinity. Because of the relatively few points available for fitting to steady state affinity models, complex models tend to give unstable fitting behavior.*

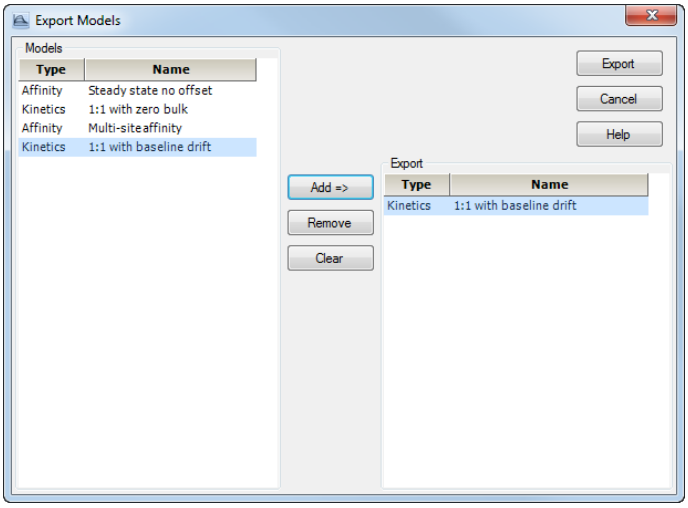
Transferring models between installations

Follow the steps below to transfer custom fitting models between different installations of the Evaluation Software and between Biacore S200 and other Biacore systems. Only custom models can be transferred.

Stage 1: Export models from the source installation

Step	Action
1	On the source installation, choose ***Tools*** →***Models*** and either ***Kinetics*** or ***Affinity*** in the submenu. It does not matter which option you choose. *Result:* The ***Models*** dialog opens. 
2	Click ***Export***. *Result:* The ***Export Models*** dialog opens, with a list of custom models on the left.
3	Select a model for export and click ***Add***.

Step	Action
	Result:
	The selected model is added to the ***Export*** list.

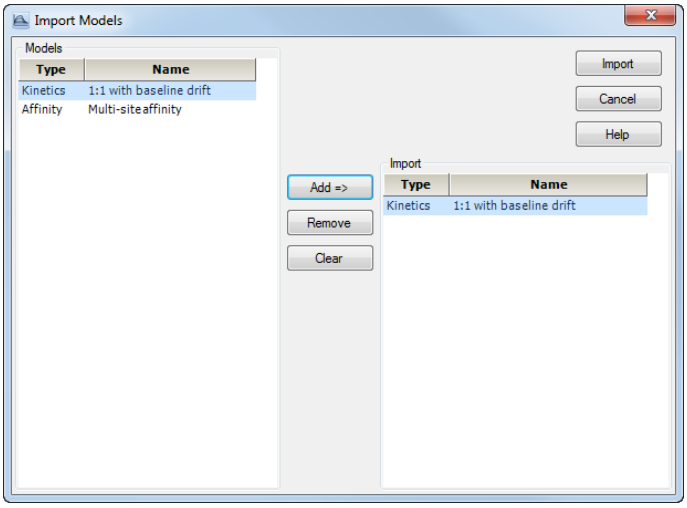


	Repeat this step for all the models you wish to transfer.
4	Click ***Export*** and provide a name for the export file.
	Result:
	The selected models are exported to an external file with extension ***.model***.

Stage 2: Import models on the destination installation

Step	Action
1	Transfer the ***.model*** file to the destination installation.
2	On the destination installation, choose ***Tools*** →***Models*** and and select the type of model you wish to import.
	Note:
	Multiple model types can be exported to a single ***.model*** *file, but only one type can be imported in one operation.*
3	Click ***Import*** and select the ***.model*** file.

Step	Action
	Result:
	The ***Import Models*** dialog opens, with a the models on the left.



4	Select a model to import export and click ***Add***.
	Result:
	The selected model is added to the ***Import*** list.
	Repeat this step for all the models you wish to transfer.
5	Click ***Import***.
	Result:
	The selected models are imported to the destination installation.

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