Biacore® T100

Software Handbook

BIACORE

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Software Handbook

Edition February 2006

Biacore® T100 Software Handbook
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Biacore AB reserves the right to make changes to the design and specification of Biacore T100 and to the content of this Handbook without prior notice. Minor differences may occur between illustrations in this Handbook and the appearance of the product.

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Contents

1. Introduction

Biacore® T100 is a high performance system for analysis of biomolecular interactions, based on Biacore's surface plasmon resonance (SPR) technology. The Control Software supplied with the system offers easy-to-use wizards for assay development and common applications together with flexible facilities for designing custom analysis methods using a graphical interface called Method Builder. Results are evaluated in separate Evaluation Software designed for efficient and flexible evaluation, with dedicated functions for common applications.

This Handbook describes in detail how to use the Control and Evaluation Software.

1.1 System overview

Instrumentation in the Biacore T100 system is described in full in the *Biacore T100 Instrument Handbook*. Important features relevant to software operation include:

- Biacore T100 supports simultaneous analysis in up to four flow cells 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 capacity) 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.

- Material that binds to the sensor surface during sample injection can be recovered in a small volume of liquid for further analysis by e.g. mass spectrometry.

- 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.

- The system includes a buffer selector valve, allowing analysis to be performed in up to four different buffers in the same unattended run.

1.2 Support for use in regulated environments

Support for use in regulated (GxP[1]) environments is provided in an optional package that adds appropriate functionality to the Biacore T100 software. Functions for GxP support are described in a separate *Biacore T100 GxP Handbook*. Descriptions of software in the current Handbook apply to installations with and without the GxP package unless otherwise stated.

1.3 Associated documentation

Biacore T100 Instrument Handbook describes the instrumentation in the Biacore T100 system, with instructions for operation, maintenance and troubleshooting.

Biacore T100 GxP Handbook describes functionality added with the optional GxP package, together with some recommendations for using the system in a regulated environment.

Other general handbooks and documentation describing the technology are available from Biacore. Information may also be found on the Internet at www.biacore.com.

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[1] GxP is used as a generic abbreviation for GLP (Good Laboratory Practice), GMP (Good Manufacturing Practice) and GCP (Good Clinical Practice).

Control Software

2. Control Software – general features

2.1 Operational modes

Biacore T100 Control Software offers three modes of operation:

- ***Manual run*** provides interactive control of the instrument operation, executing commands singly 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.

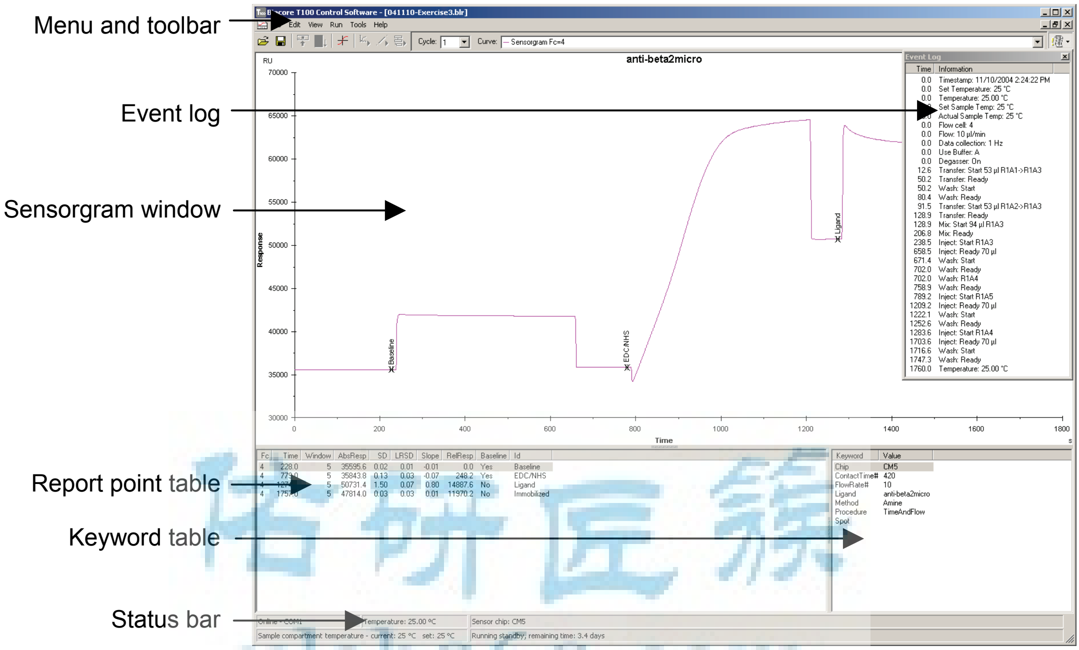
- ***Application wizards*** provide guidance in setting up experiments for assay development and execution. Separate wizards are offered for different purposes such as ligand immobilization, concentration determination or measurement of kinetic constants. Each wizard consists of an ordered series of dialog boxes, ensuring that the essential features of the application setup are correctly defined.

- ***Methods*** provide greater flexibility (and conversely less guidance) in setting up applications, allowing customized applications that are not covered by wizards. Methods are defined in a graphical interface called ***Method Builder***, which is designed to provide full flexibility in method definition while retaining a simple interface for running assays based on established methods. Application wizard templates may be opened in Method Builder to provide a starting point for further refinement of application setup. Predefined methods are also provided by Biacore as help in defining methods for selected purposes.

Each of these modes of operation is described in more detail in the following chapters.

2.2 User interface

The main screen in the control software is divided into 5 main areas with a separate event log window:



```
Time    Information
   0.0  Timestamp: 11/10/2004 2:24:22 PM
   0.0  Set Temperature: 25 °C
   0.0  Temperature: 25.00 °C
   [illegible]  Set Sample Temp: 25 °C
   0.0  Actual Sample Temp: 25 °C
   0.0  Flow cell: 4
   0.0  Flow: 10 µl/min
   0.0  Data collection: 1 Hz
   0.0  Use Buffer: A
   0.0  Degasser: On
  12.6  Transfer: Start 53 µl R1A1->R1A3
  50.2  Transfer: Ready
  50.2  Wash: Start
  80.4  Wash: Ready
  91.5  Transfer: Start 53 µl R1A2->R1A3
 128.9  Transfer: Ready
 128.9  Mix: Start 94 µl R1A3
 206.8  Mix: Ready
 238.5  Inject: Start R1A3
 658.5  Inject: Ready 70 µl
 671.4  Wash: Start
 702.0  Wash: Ready
 702.0  Wash: R1A4
 758.9  Wash: Ready
 789.2  Inject: Start R1A5
1209.2  Inject: Ready 70 µl
1222.1  Wash: Start
1252.6  Wash: Ready
1283.6  Inject: Start R1A4
1703.6  Inject: Ready 70 µl
1716.6  Wash: Start
1747.3  Wash: Ready
1760.0  Temperature: 25.00 °C
```

Fc	Time	Window	AbsResp	SD	LRSD	Slope	RelResp	Baseline	Id
4	228.0	5	35595.6	0.02	0.01	-0.01	0.0	Yes	Baseline
4	779.0	5	35843.8	0.13	0.03	-0.07	248.2	Yes	EDC/NHS
4	[illegible]	5	50731.4	1.50	0.07	0.80	14887.6	No	Ligand
4	1757.0	5	47814.0	0.03	0.03	0.01	11970.2	No	Immobilized

Keyword	Value
Chip	CM5
ContactTime#	420
FlowRate#	10
Ligand	anti-beta2micro
Method	Amine
Procedure	TimeAndFlow
Spot	

Online - COM1 | Temperature: 25.00 °C | Sensor chip: CM5

Sample compartment temperature - current: 25 °C set: 25 °C | Running standby, remaining time: 3.4 days

- The *menu* and *toolbar* provide access to control commands

- The *sensorgram window* displays the sensorgrams for the current run or the currently open file

- The *report point table* lists report points for the currently displayed cycle. Report points are defined automatically: custom report points can also be added in methods.

- The *keyword table* lists keywords for the currently displayed cycle. Keywords are defined automatically in wizard runs, or in the method for method runs.

- The *status bar* displays the instrument status, including the temperature of the detector and the sample compartment and the type of chip docked.

- 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.

2.2.1 On-line help

On-line help is available at any time from the **Help** menu. Context-specific help for dialog boxes is provided through **Help** buttons in the boxes.

2.3 Basic operation

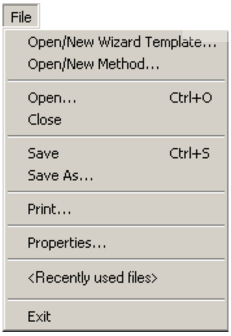
2.3.1 Selecting cycles and sensorgrams

During a run, the current cycle is displayed by default. You can choose which cycle to display in the **Cycle** selector, but the display will revert to the current cycle when a new cycle is started. For a completed run, choose which cycle to display with the **Cycle** selector in the toolbar:

Cycle: 13 Curve: Subtracted Fc=3-1

The **Curve** selector determines which curve in the cycle is current. Options in the **View** menu (Section 2.3.4) control which curves are displayed in the sensorgram window.

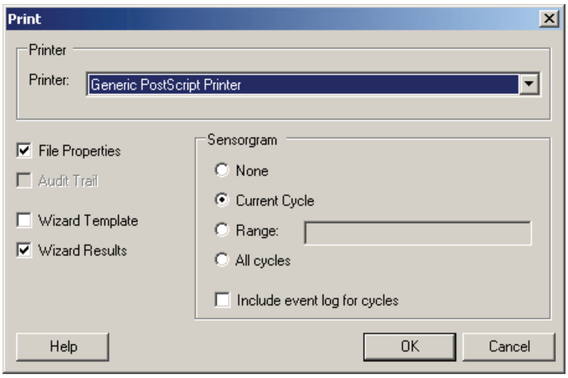
2.3.2 File menu



The **Open/New** options for wizard templates and methods create new wizard templates and methods, and open existing templates and methods for editing or for starting a run.

Open opens result files. Most result files just display the sensorgrams and tables. Files from immobilization and regeneration scouting wizards also display a summary window showing the results of the run (see Sections 4.3.2 and 4.4.1).

Print prints a hard-copy of the results. Select the printer to use and check the items you wish to print.



Print

Printer

Printer: Generic PostScript Printer

☑ File Properties

☐ Audit Trail

☐ Wizard Template

☑ Wizard Results

Sensorgram

○ None

● Current Cycle

○ Range:

○ All cycles

☐ Include event log for cycles

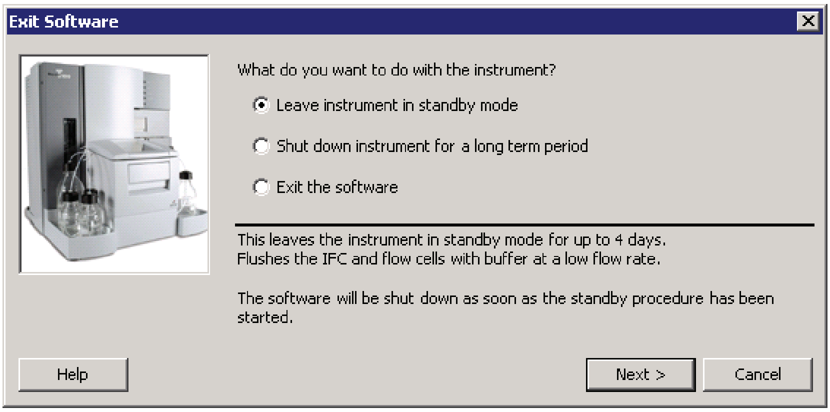
Sensorgrams will be printed as follows:

None	No sensorgrams will be printed
Current cycle	The current cycle will be printed with the **View:Show...** setting and scale as shown on the screen.
Range and **All cycles**	Multiple cycles will be printed. For **Range**, enter a range or cycle numbers separated by commas (e.g. **4-16,19,22**). All curves will be included in each cycle regardless of the **View:Show...** setting. Sensorgrams will be printed at full scale unless the **Lock Scale** box is checked in the sensorgram window, in which case the current scaling will be applied to all cycles (with this setting, some sensorgrams may appear to be empty).

Note: In order to maintain report layout, the print orientation is fixed regardless of the printer settings in Windows.

Properties shows detailed properties of the currently opened run, including the properties of the sensor chip used in the run.

When you close the software with **Exit** while the instrument is still switched on, you may choose to shut down the instrument for a shorter or longer period if required.



Exit Software

What do you want to do with the instrument?

● Leave instrument in standby mode

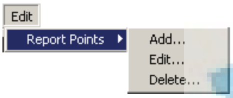
○ Shut down instrument for a long term period

○ Exit the software

This leaves the instrument in standby mode for up to 4 days.
Flushes the IFC and flow cells with buffer at a low flow rate.

The software will be shut down as soon as the standby procedure has been started.

2.3.3 Edit menu

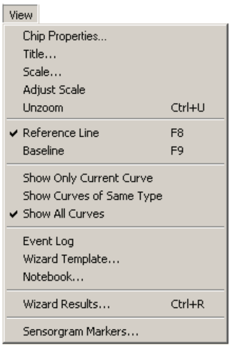


Options in the **Edit** menu allow you to add, edit and delete report points. Report points are created automatically and are used in various evaluation contexts. You should in general avoid editing or deleting report points that are created automatically.

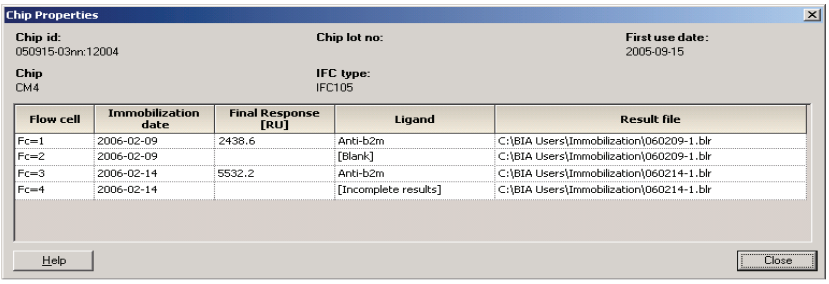
Editing operations for report points in the Control Software may be applied to single curves or to all curves in the current cycle. Note that editing operations are *not* applied to multiple cycles.

Report points created in the Control Software cannot be edited in the Evaluation Software. The Evaluation Software offers functions for creating and editing custom report points that can be applied to all cycles in the run in a single operation. This is usually preferable to adding report points in the Control Software.

2.3.4 View menu



Chip Properties opens a dialog box that displays the properties of the currently docked sensor chip. The **Ligand** column is empty for flow cells that have not been used, and shows **[Blank]** for flow cells that have been prepared as a blank reference surface by activation and deactivation. The text **[Incomplete results]** indicates that the immobilization run was interrupted (by for instance user intervention or power failure) before it could be completed.



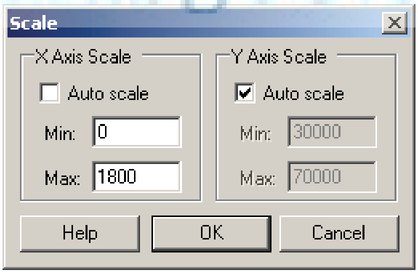
Chip id: 050915-03nn:12004
Chip lot no:
First use date: 2005-09-15
Chip CM4
IFC type: IFC105

Flow cell	Immobilization date	Final Response [RU]	Ligand	Result file
Fc=1	2006-02-09	2438.6	Anti-b2m	C:\BIA Users\Immobilization\060209-1.blr
Fc=2	2006-02-09		[Blank]	C:\BIA Users\Immobilization\060209-1.blr
Fc=3	2006-02-14	5532.2	Anti-b2m	C:\BIA Users\Immobilization\060214-1.blr
Fc=4	2006-02-14		[Incomplete results]	C:\BIA Users\Immobilization\060214-1.blr

Properties for the sensor chip used in a currently open run may be found under **File:Properties** (Section 2.3.2).

Title sets a title in the sensorgram window. The default title is the assay step name.

Scale sets the scale of the sensorgram window:



X Axis Scale
☐ Auto scale
Min: 0
Max: 1800

Y Axis Scale
☑ Auto scale
Min: 30000
Max: 70000

If you set **Auto scale**, the scale will be adjusted if necessary to accommodate the full data range of the currently displayed cycle. During a run, the scale is adjusted at intervals as more data is collected. Check the **Lock scale** box in the top right corner of the sensorgram window to lock the scale to the current settings.

Adjust Scale sets the scale to the full data range. This will not affect the **Auto scale** setting in the **Scale** dialog. **Adjust Scale** overrides but does not turn off the **Lock scale** setting.

To scale the sensorgram display interactively, drag with the cursor over the area to be scaled. Double-clicking in the display or choosing **View:Unzoom** restores the previous zoom setting.

Reference line toggles display of a movable vertical line in the sensorgram window, together with a separate small window that shows the response and time coordinates at the reference line for the current curve. Use the **Curve** selector in the toolbar (see Section 2.3.1) to set the current curve. Drag the reference line to move it. When the reference line is displayed, choosing **Baseline** sets a baseline at the current reference line position, and the coordinates window shows the response relative to that baseline.

The options **Show Only Current Curve, Show Curves of Same Type** and **Show All Curves** control which curves are displayed in the sensorgram window. Curve types distinguish between unsubtracted and reference-subtracted curves.

Choose the **Event Log** option or click on the **Event Log** button at the right of the toolbar to display the event log window.

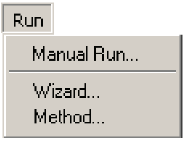
Choose the **Wizard Template** or **Method** options to display the wizard or method definition for the run. You can edit the definition and save it as a new wizard template or method. You cannot however change the original definition that is saved together with the result file.

Notebook opens a notebook window where details of the run may be recorded. The notebook is only available during a run or for a completed result file.

For immobilization and regeneration scouting wizard runs, the **Wizard Results** option opens a window showing the results of the run. All other runs are evaluated in the Evaluation Software.

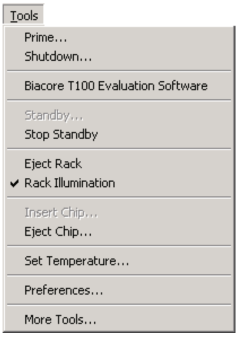
Sensorgram Markers controls display of report point and event markers and labels in the sensorgram window.

2.3.5 Run menu



The options in the **Run** menu are used to start the different types of runs (see Chapter 3 and Sections 4.1.2 and 5.9.7).

2.3.6 Tools menu



Options in the **Tools** menu control instrument operations outside the context of runs.

Prime flushes the flow system with fresh buffer. There is an option to include **Prime** at the beginning of each wizard- or method-based run. Use the menu option when you want to flush the system at other times (e.g. before a manual run).

Shutdown starts the procedure for shutting down the instrument for long periods of time (more than 4 days). The procedure displays necessary instructions on the screen. Details of the shutdown procedure are given in the Biacore T100 Instrument Handbook.

Standby puts the instrument in standby mode, which maintains a low buffer or water flow through the flow system for up to 4 days. Leaving the instrument in standby mode when not in use is generally recommended. The instrument is automatically put in standby mode at the end of a run. Use the menu option if standby has been stopped and you want to restart it.

Stop Standby stops standby mode.

Eject Rack ejects the rack tray from the sample compartment. The rack may be ejected during setup for wizard- and method-based runs, and at any time during a manual run. Use the menu command when you want to eject the rack at any other time.

> *WARNING!*
> **The rack tray automatically moves into the instrument 60 seconds after it has been ejected. A timer in the dialog indicates when the rack tray will be automatically moved into the instrument.**

Rack Illumination switches the blue illumination in the sample compartment on or off. The illumination helps you to see in the sample compartment but does not otherwise affect instrument function.

Insert Chip and **Eject Chip** are used for docking and undocking the sensor chip respectively. More details are given in Chapter 3 of the Biacore T100 Instrument Handbook.

Set Temperature sets the sample compartment and analysis temperature. More details are given in Chapter 3 of the Biacore T100 Instrument Handbook.

Preferences controls aspects of file storage and data import (see Section 2.4).

More Tools provides access to maintenance, test and service tools. Details are given in Appendix B of the Biacore T100 Instrument Handbook.

2.3.7 Right-click menus

Right-clicking with the mouse in some windows opens context menus specific for the window.

Sensorgram window

Scale opens the same dialog as the **View:Scale** option (Section 2.3.4).

Copy Graph copies the sensorgram window exactly as displayed to the Windows clipboard. Use this option to insert a copy of the sensorgram window into other programs such as presentation software.

Export Curves exports data for the currently displayed curves to a text file. Entire curves are exported regardless of the scale of the display. The exported data includes report points and event marker times if these are displayed in the sensorgram window. See Appendix A for more details of the export format.

Gridlines toggles display of gridlines in the sensorgram window.

Report point table

The right-click menu options for the report point table correspond to the **Edit:Report Points** menu options.

Notebook

Right-click menu options in the notebook represent standard Windows editing functions.

2.4 File storage

2.4.1 *Wizard templates and methods*

Wizard templates are saved in files with a file name extension **.bw****, where ****** represents an abbreviation that identifies the wizard (e.g. a wizard template for concentration analysis has the extension **.bwConc**).

Methods are saved in files with the file name extension **.Method**.

Note: The extension will not be displayed if the default setting **Hide file extensions for known file types** is selected in the Windows Explorer folder options. Turning this setting off can help you to identify file types in dialog boxes.

Templates and methods may be saved in any location. A folder structure under the default location as specified in **Tools:Preferences** is however recommended, since files in this location are handled preferentially in the **Open/New** dialog boxes for wizards and templates (see Section 4.1.1).

...

Click on the browse button to change the folder setting.

2.4.2 *Result files*

Results are saved in files with the file name extension **.blr**. Result files from wizard- or method-based runs contain a copy of the wizard template or method as well as the results of the run.

3. Manual run

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.

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 the tools for concentration, kinetics/affinity, thermodynamics or affinity in solution.

3.1 Preparing for a manual run

3.1.1 Instrument preparations

The integrated instrument preparation steps that are included with wizard- and method-based runs are not supported for manual run. The instrument should therefore be prepared using options from the **Tools** menu.

1. Dock the chip that you want to use, and immobilize ligand on the surface (see Section 4.3.2) if this has not already been done.

2. Choose **Tools:Prime** to flush the flow system with fresh buffer.

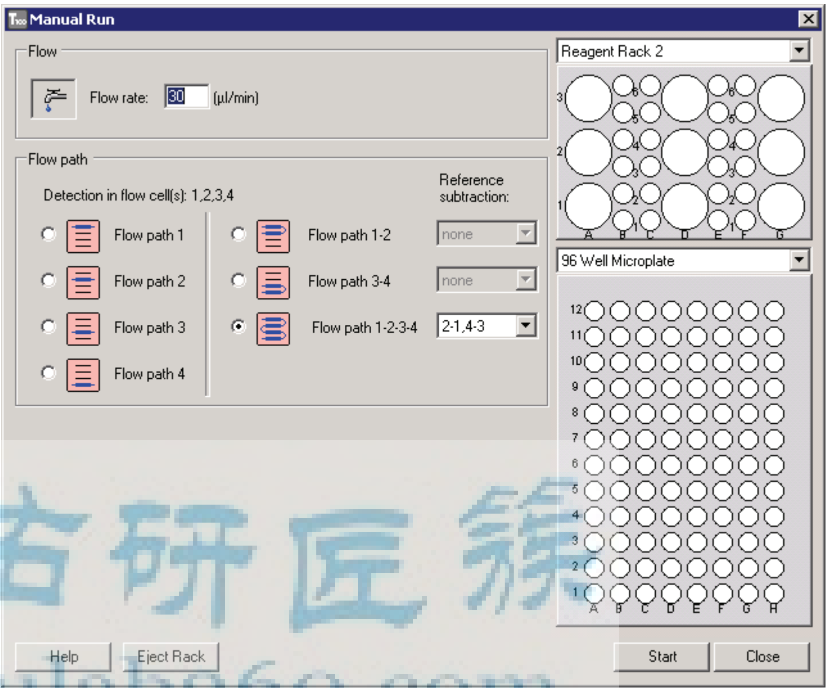
3. Choose **Normalize** from the **Maintenance Tools** section of **Tools:More Tools** if the detector has not been normalized since the chip was docked. (In many cases, the detector will have been normalized in connection with ligand immobilization. However, you may need to perform the operation again if the chip has been undocked and re-docked after immobilization.)

4. Choose **Tools:Set Temperature** and set the analysis and sample compartment temperatures. Wait until the analysis temperature is stable (as shown in the status bar) before starting the run.

5. Prepare your samples and reagents in the microplate and/or reagent rack. Note the rack positions and volumes of samples that you prepare: there is no software support in manual run for identifying samples or monitoring the volume of liquid in the autosampler positions. You insert the samples as part of the starting procedure for the run. You can also add samples during the run.

3.2 Starting a manual run

Choose **Run:Manual Run** to start a manual run.



Flow rate: 30 (µl/min)

Detection in flow cell(s): 1,2,3,4

● Flow path 1-2-3-4 2-1,4-3

Reagent Rack 2

96 Well Microplate

Choose the initial settings for flow rate and flow path with 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.

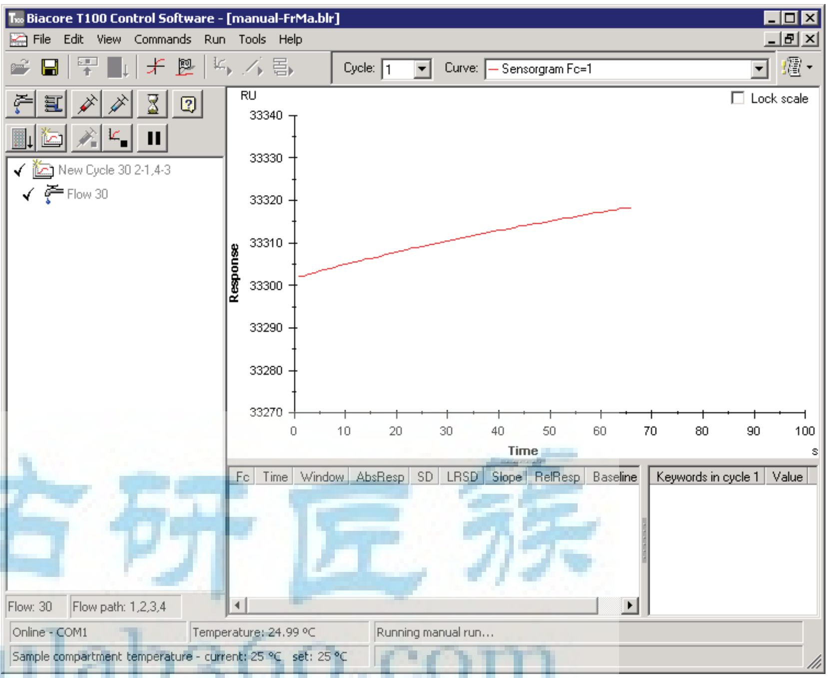
Choose the rack and microplate settings. These will apply throughout the run and cannot be changed.

Click **Eject Rack** to eject the rack tray so that you can load your samples.

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

Control the manual run from the command buttons in the main window or the options in the **Command** menu:



Flow: 30

Flow path: 1,2,3,4

Online - COM1

Temperature: 24.99 °C

Running manual run...

Sample compartment temperature - current: 25 °C set: 25 °C

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.

Right-click on a pending command for 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 command or commands and paste them elsewhere in the queue. The **Copy** function works with both completed and pending commands.

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 inject

Injects sample. 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 inject

Injects regeneration solution. Choose the position from which the solution 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 solution for the injection. The required volume for the specified contact time is indicated in the dialog box.

Check **High viscosity solution** 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.

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, rather than at the end of the queue, 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.

WARNING!
The rack tray automatically moves into the instrument 60 seconds after it has been ejected. A timer in the dialog indicates when the rack tray will be automatically moved into the instrument.

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.

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

To end a manual run:

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 **Tools:Eject Rack** to eject the rack tray and remove your samples and reagents.

3. Choose **Tools:Eject Chip** to undock the chip if desired.

4. Application wizards

Application wizards guide you through the procedure of setting up common applications, with recommendations and settings based on Biacore's expertise in the field of SPR-based interaction studies. Wizards are an ideal starting point for inexperienced or infrequent users, since they offer a structured sequence of settings that covers all essential aspects of the assay in question. Wizard settings can be saved in ***templates*** for later use. Advanced users can open wizard templates in Method Builder for more flexible assay design (see Chapter 5).

4.1 Wizard templates

An application wizard consists of a series of dialog boxes that takes you through the steps in setting up the application. Settings in the dialog boxes may be saved in ***wizard templates***, so that opening a template will present the saved settings in each dialog box.

Normally, a wizard template is saved when all steps have been defined, so that the template represents a complete assay definition including sample details if desired. If a wizard sequence is closed before reaching the last step, however, you are given an opportunity to save the template, which will then contain settings as far as they have been defined.

4.1.1 Creating and editing wizard templates

To create a new wizard template or edit an existing template, choose **File:Open/New Wizard Template** and select the type of wizard in the dialog box. Click **New** to create a new template, or navigate to the folder where your template is stored, select the template and click **Open** to edit an existing template.

The top-level folder for wizard templates is defined under **Tools: Preferences** (see Section 2.4). You can navigate between subfolders under the top level in the dialog box, but you cannot access files outside the top-level folder from within the dialog box. Click **Browse** to navigate freely in the computer file structure and open wizard templates stored in other locations.

Note: The **Open/New Wizard Template** dialog box only lists templates of the selected type, but the **Browse** dialog may list all types. Template types are identified by the file extension, which may or may not be displayed according to your Windows Explorer settings (see Section 2.4.1).

4.1.2 Running wizards

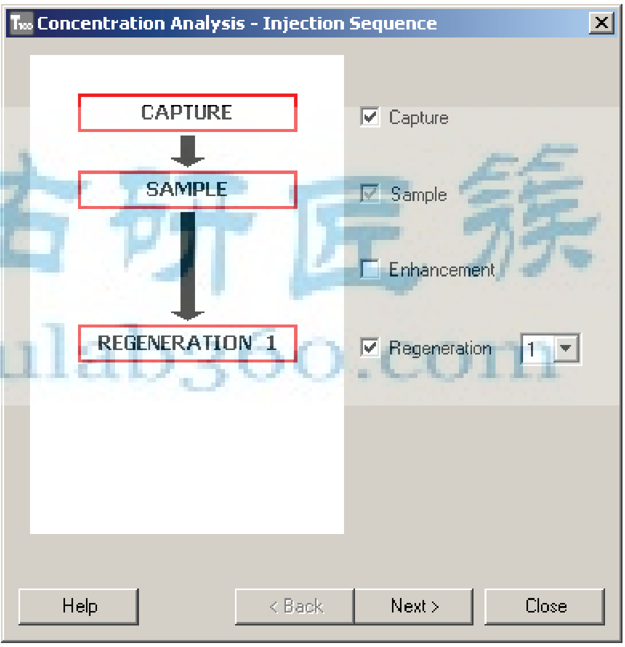
When you start a run based on a wizard template, you step through the dialog boxes in the template and may change settings if desired.

4.2 Common wizard components

Several dialogs are common to a number of wizards, with equivalent functions and only minor differences if any. These steps are described in this section. Any wizard-specific variations in these common components are described in the section on the appropriate wizard below.

4.2.1 Injection sequence

This dialog determines the sequence of injections in the wizard analysis cycle. Some injections are not supported in certain wizards (e.g. the kinetics wizard does not support enhancement injections).



☑ Capture
☑ Sample
☐ Enhancement
☑ Regeneration 1

Check the injections that you want to include. The illustration panel shows the sequence of included injections. Injections have the following purposes:

Capture
Intended for ligand solution in applications that use a capturing approach to attach the ligand to the surface. The same solution will be used for the capture injection in all cycles: you cannot vary the captured ligand within the context of one wizard run.

The flow path for capture solution depends on the settings for detection (see Section 4.2.2).

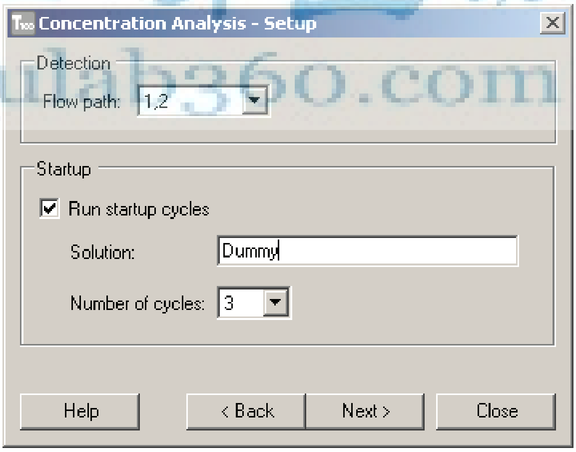
Sample
This is the sample to be analyzed. The solution used for the sample injection is normally different in different cycles, and is specified in the sample table at a later stage in the wizard. The sample injection is required in all wizards.

Enhancement
Intended for injection of a secondary reagent that binds to analyte on the surface, typically used either to amplify the response obtained from the analyte or to enhance the specificity of analyte detection. The same solution will be used for the enhancement injection in all cycles.

Regeneration
One or two regeneration injections may be included, which may use the same or different solutions. The regeneration procedure is the same in all cycles.

4.2.2 Assay setup

Common features of the assay setup dialog are choice of detection and flow path and specification of start-up cycles at the beginning of the run.



Concentration Analysis - Setup

Detection

Flow path: 1,2

Startup

☑ Run startup cycles

Solution: Dummy

Number of cycles: 3

Flow path
Select the flow path for the analysis. The setting will apply throughout the whole wizard run.

The flow paths available vary between the different wizards.

The detection is automatically set to the same settings as the flow path, so that sensorgrams are recorded only from the flow cells used.

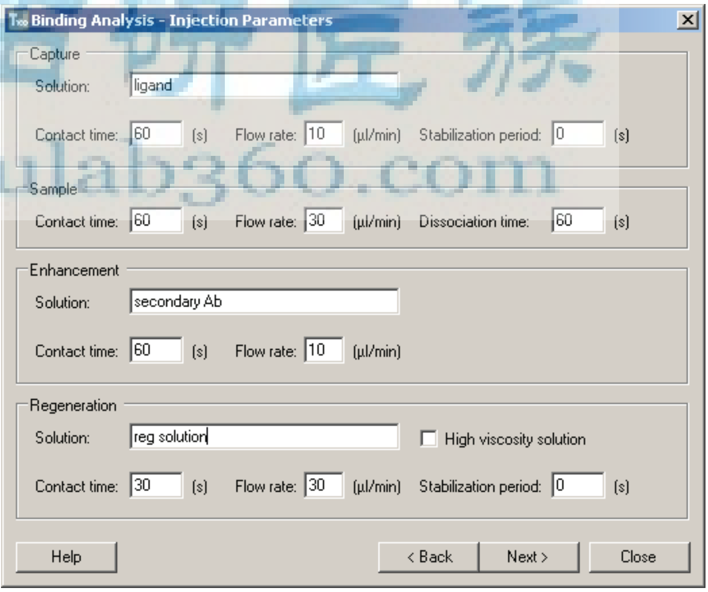
When reference subtraction is used together with ligand capture (Section 4.2.1), the captured ligand passes over the active surface but not the reference surface (for example, with **Flow path** set to **2-1**, the ligand is injected in flow cell 2 but not flow cell 1). If the **Flow path** setting does not use reference subtraction, ligand is injected in all flow cells included in the flow path.

Start-up cycles

Start-up cycles are identical to analysis cycles except that the sample is replaced by a dummy sample. The response from a newly prepared or newly docked sensor chip often shows some instability during the first few cycles, and start-up cycles allow the response to stabilize before the first analysis cycle is performed. Three start-up cycles are generally recommended for most assay purposes, to ensure a stable response in the analysis. Start-up cycles are treated separately from analysis cycles in the evaluation software.

4.2.3 Injection parameters

The **Injection parameters** dialog specifies details of the injections selected in the **Injection sequence**.



Binding Analysis - Injection Parameters

Capture
Solution: ligand
Contact time: 60 (s) Flow rate: 10 (µl/min) Stabilization period: 0 (s)

Sample
Contact time: 60 (s) Flow rate: 30 (µl/min) Dissociation time: 60 (s)

Enhancement
Solution: secondary Ab
Contact time: 60 (s) Flow rate: 10 (µl/min)

Regeneration
Solution: reg solution
☐ High viscosity solution
Contact time: 30 (s) Flow rate: 30 (µl/min) Stabilization period: 0 (s)

Details of this dialog box may vary according to the injections selected and the particular wizard. Some features may be generalized:

Parameter limits

Flow rates can be set between 1 and 100 µl/min in increments of 1 µl/min.

Permitted ranges for injection contact times are determined by the flow rate together with the limits for injected volumes, which are 2–350 µl for normal solutions and 5-100 µl for viscous regeneration solutions (see below).

Note: The injected volume of solution is determined by the combination of flow rate and contact time, rounded up to the nearest whole number. At low flow rates, this can result in contact times that are longer than requested: for example, at 1 µl/min a requested contact time of 90 s (requiring 1.5 µl solution) will result in an actual contact time of 120 s (solution volume rounded up to 2 µl).

Stabilization time after injection

This function is available after a capture injection and after the last injection in the sequence. For capture injections, a stabilization time can be useful if a fraction of the ligand dissociates rapidly. Including a stabilization time to allow for such dissociation can help to improve reproducibility.

A stabilization time may be used after the last injection instead of regeneration for systems where analyte dissociates rapidly and completely from the surface.

Exposure of the surface to regeneration solution can often lead to transient changes in the baseline. Inclusion of a stabilization time after regeneration helps to ensure a stable baseline for the next cycle.

Sample injection

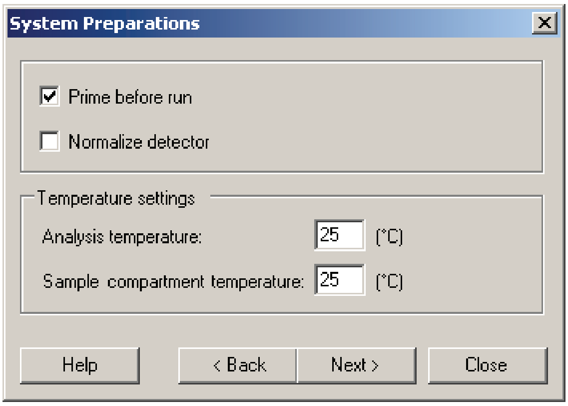
Normally, the injected sample solution is specified in a separate sample table. Some wizards (e.g. **Surface Performance**) use only a single sample solution that is specified together with the other injection parameters in this dialog box.

Regeneration

The parameters for regeneration include a check-box for **High viscosity solution.** Check this box 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 modify the injection procedure for better handling of viscous solutions. The maximum injected volume is limited to 100 µl for viscous solutions.

4.2.4 System preparations

This dialog box specifies how the system will be prepared before the first cycle.



System Preparations

☑ Prime before run

☐ Normalize detector

Temperature settings

Analysis temperature: 25 (°C)

Sample compartment temperature: 25 (°C)

Prime before run

This option flushes the flow system with running buffer to make sure that all buffer is fresh. You should generally prime the system before each run to ensure fresh buffer throughout the flow system.

Normalize

This option adjusts the detector response to compensate for small variations in reflectance characteristics between individual sensor chips. For best results, you should normalize the detector whenever the chip is changed. You do not need to run normalization if the same chip remains docked between runs. Normalization injects BIAnormalizing solution (70% glycerol) over the surface: if your ligand does not withstand exposure to this solution, normalize the detector before you run ligand immobilization.

Analysis temperature

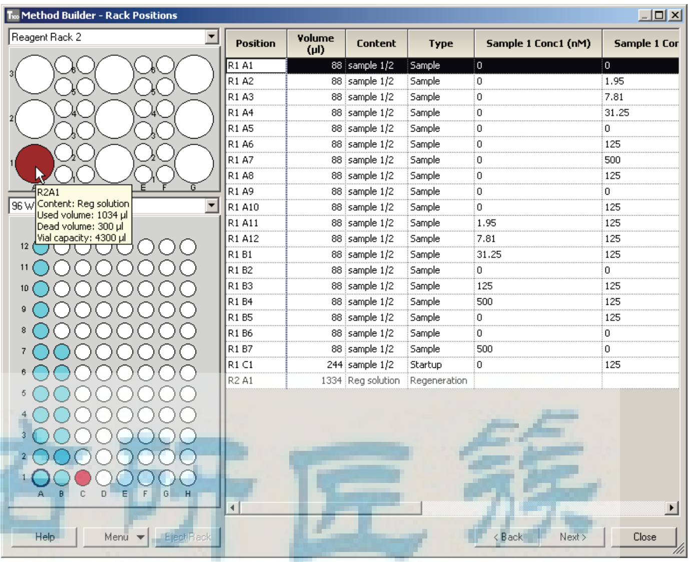
This is the temperature at the flow cell. If the specified value differs from the current temperature, the system will wait at the beginning of the run until the analysis temperature is stable at the new value. You can choose to ignore temperature instability, but the response will drift as the temperature stabilizes. The absolute response decreases by about 150 RU for a 1°C increase in temperature.

Sample compartment temperature

This is the temperature in the sample compartment. Equilibration of the sample compartment to a new temperature will start when the run is started. The system will not wait for a stable sample compartment temperature at the beginning of the run: samples equilibrate to the analysis temperature during passage through the IFC, so that the sample compartment temperature is not critical for the measured SPR response.

4.2.5 Rack positions

This dialog box shows where samples and reagents are placed in the microplate and rack. Positions are color-coded according to sample and reagent categories: you can change the color-coding in the **Automatic positioning** dialog, accessed through the **Menu** button.



R2A1
Content: Reg solution
Used volume: 1034 µl
Dead volume: 300 µl
Vial capacity: 4300 µl

Position	Volume (µl)	Content	Type	Sample 1 Conc1 (nM)	Sample 1 Cor
R1 A1	88	sample 1/2	Sample	0	0
R1 A2	88	sample 1/2	Sample	0	1.95
R1 A3	88	sample 1/2	Sample	0	7.81
R1 A4	88	sample 1/2	Sample	0	31.25
R1 A5	88	sample 1/2	Sample	0	0
R1 A6	88	sample 1/2	Sample	0	125
R1 A7	88	sample 1/2	Sample	0	500
R1 A8	88	sample 1/2	Sample	0	125
R1 A9	88	sample 1/2	Sample	0	0
R1 A10	88	sample 1/2	Sample	0	125
R1 A11	88	sample 1/2	Sample	1.95	125
R1 A12	88	sample 1/2	Sample	7.81	125
R1 B1	88	sample 1/2	Sample	31.25	125
R1 B2	88	sample 1/2	Sample	0	0
R1 B3	88	sample 1/2	Sample	125	125
R1 B4	88	sample 1/2	Sample	500	125
R1 B5	88	sample 1/2	Sample	0	125
R1 B6	88	sample 1/2	Sample	0	0
R1 B7	88	sample 1/2	Sample	500	0
R1 C1	244	sample 1/2	Startup	0	125
R2 A1	1334	Reg solution	Regeneration		

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

Positions are described by tool tips (place the cursor on the position for a couple of seconds to display the tool tip). Empty positions show the position capacity and dead volume. Used positions show in addition the content name and the volume that will be used.

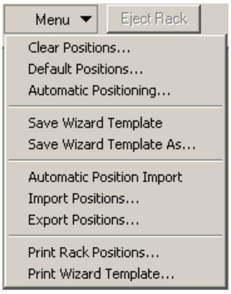
You can change sample and reagent positions manually in two ways:

- 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.
- Type a position directly in the **Position** column in the table, or copy (Ctrl-C) a position from one row and paste it (Ctrl-V) in another.

Positions can also be reorganized using the **Automatic positioning** dialog (see below).

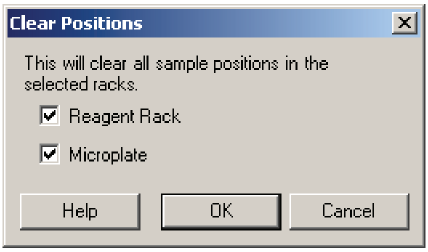
Menu functions

Use the **Menu** button to access additional functions for rack positioning.



Clear Positions

This option clears the entries in the **Positions** column for the selected rack or plate.


Clear Positions

This will clear all sample positions in the selected racks.

☑ Reagent Rack

☑ Microplate

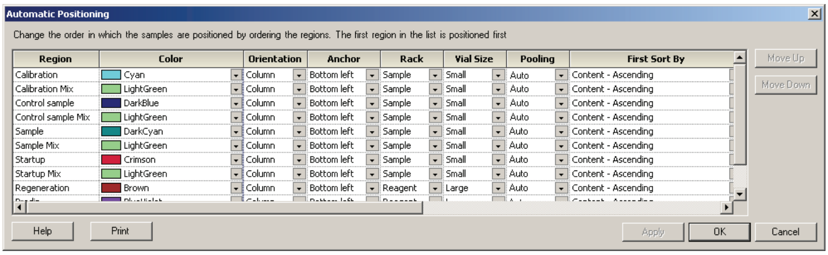
Positions that are cleared must be reassigned before the run can be started.

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 wizard template.

Automatic Positioning

This option controls the way samples and reagents are positioned automatically. Samples and reagents are placed by category, and categories are kept contiguous as far as possible.


Automatic Positioning

Change the order in which the samples are positioned by ordering the regions. The first region in the list is positioned first

Region	Color	Orientation	Anchor	Rack	Vial Size	Pooling	First Sort By
Calibration	Cyan	Column	Bottom left	Sample	Small	Auto	Content - Ascending
Calibration Mix	LightGreen	Column	Bottom left	Sample	Small	Auto	Content - Ascending
Control sample	DarkBlue	Column	Bottom left	Sample	Small	Auto	Content - Ascending
Control sample Mix	LightGreen	Column	Bottom left	Sample	Small	Auto	Content - Ascending
Sample	DarkCyan	Column	Bottom left	Sample	Small	Auto	Content - Ascending
Sample Mix	LightGreen	Column	Bottom left	Sample	Small	Auto	Content - Ascending
Startup	Crimson	Column	Bottom left	Sample	Small	Auto	Content - Ascending
Startup Mix	LightGreen	Column	Bottom left	Sample	Small	Auto	Content - Ascending
Regeneration	Brown	Column	Bottom left	Reagent	Large	Auto	Content - Ascending

Region	This column lists the sample and reagent categories for the run.
Color	This option controls the display color for the category.
Orientation	This column determines whether samples are arranged by **column** (vertically in the rack and plate diagram) or **row** (horizontally in the diagram).

Anchor	This column determines the position for the first sample in the category.
Rack	This option 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 category.
Vial size	Use this option to determine the vial size for reagents. If **Auto** is chosen, placement is decided on the basis of the volume of solution.
Pooling	This option allows you to combine solutions with the same name 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 Choose **Auto** to set the pooling according to the type of region.
Sort by	Solutions within a category 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.

Save Wizard Template/Save Wizard Template As

Saves the wizard template, with either the same or a different file name. The corresponding function is also available for methods.

Automatic Position Import

This option imports positioning information from an external source. The option is only available if **Enable automatic rack positions import** is checked in **Tools:Preferences**. 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 for more details.

Import Positions

Imports positioning details from an external file. Details of the import settings and file format are described in Appendix A.

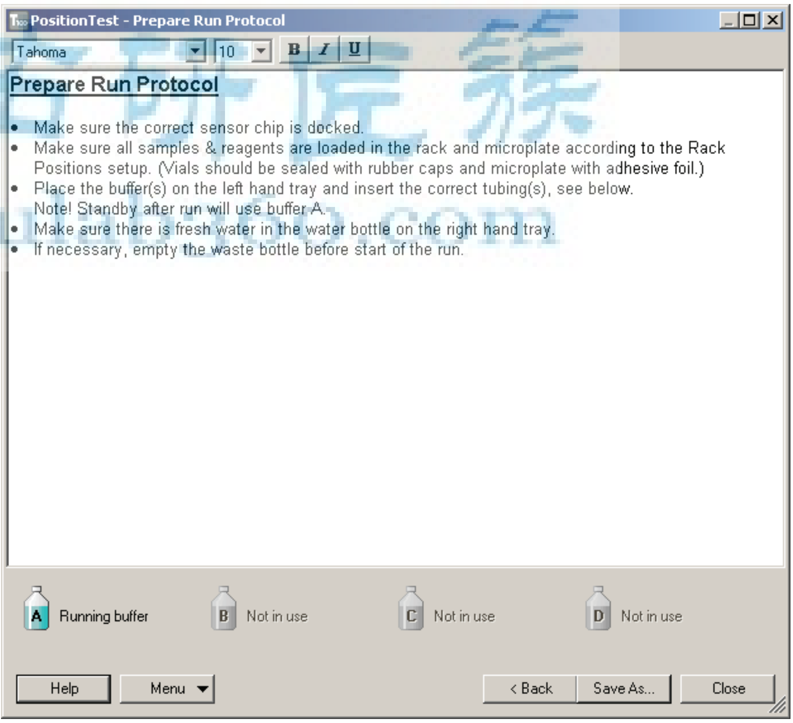
Export Positions
Exports the data in the positioning table to a tab-separated text file. See Appendix A for details of the exported file format.

Print Rack Positions
Prints a copy of the rack positions diagram and table.

Print Wizard Template/Print Method
Prints a copy of the currently open wizard template or method.

4.2.6 Prepare Run protocol

This dialog box allows you to enter a run protocol to provide instructions to the user when the run is started. A suggested general protocol is provided.



Prepare Run Protocol

- Make sure the correct sensor chip is docked.
- Make sure all samples & reagents are loaded in the rack and microplate according to the Rack Positions setup. (Vials should be sealed with rubber caps and microplate with adhesive foil.)
- Place the buffer(s) on the left hand tray and insert the correct tubing(s), see below.
  Note! Standby after run will use buffer A.
- Make sure there is fresh water in the water bottle on the right hand tray.
- If necessary, empty the waste bottle before start of the run.

Use the controls at the top of the dialog box to set font characteristics for the text.

The area at the bottom of the dialog box identifies the buffers required in each of the buffer bottles. For all wizards except buffer scouting, only buffer A is used. For the buffer scouting wizard (Section 4.4.3) and Method Builder-based runs (Section 5.4), buffer names are shown in the **Prepare Run Protocol** dialog.

The **Menu** button provides options for saving and printing the wizard template (Section 4.2.5).

4.3 Surface preparation wizards

4.3.1 Immobilization pH scouting

The **Immobilization pH scouting** wizard helps you to find the optimal pH for immobilizing your ligand, by testing ligand pre-concentration at a range of pH values. See the Biacore Sensor Surface Handbook for further details. The injection sequence for immobilization pH scouting is fixed.

1. *Assay setup*

Flow path: 1

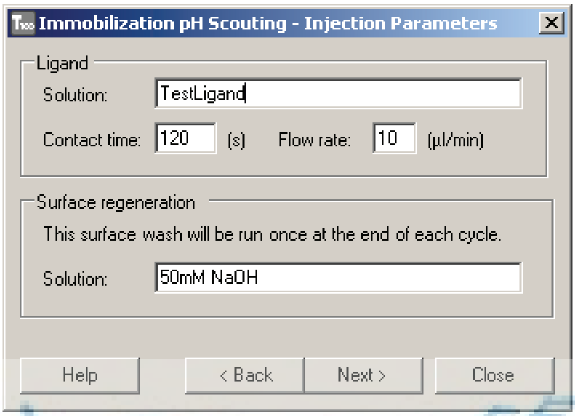
	Buffer Name	pH
1	10 mM Acetate	5.5
2	10 mM Acetate	5
3	10 mM Acetate	4.5
4	10 mM Acetate	4
5		

Choose the flow path for the pH scouting. Immobilization pH scouting is restricted to a single flow cell within a run. The sensor surface in the flow cell should be unmodified.

Enter the buffers and pH values to be used for scouting. The default list covers sodium acetate buffers in the pH range 4 to 5.5, available as ready-to-use solutions from Biacore. Buffers will be tested in the order listed.

Note: The buffers listed here are buffers in which the ligand should be prepared. They are not used as running buffers: you should use the same running buffer for pH scouting as you intend to use during immobilization.

2. *Injection parameters*


Immobilization pH Scouting - Injection Parameters

Ligand

Solution: TestLigand

Contact time: 120 (s) Flow rate: 10 (µl/min)

Surface regeneration

This surface wash will be run once at the end of each cycle.

Solution: 50mM NaOH

Enter the name of the ligand to be tested and the contact time and flow rate. A recommended contact time is 120 seconds: you may need to use a longer contact time if preconcentration of ligand on the sensor surface proves to be slow.

The surface is washed with a “regeneration” injection at the end of each cycle to remove any ligand that might remain on the surface. The recommended solution for this procedure is 50 mM NaOH.

3. *System preparations*

See Section 4.2.4. Run immobilization pH scouting at the same temperature as you intend to run the immobilization (electrostatic preconcentration is however usually fairly insensitive to temperature).

4. *Rack positions*

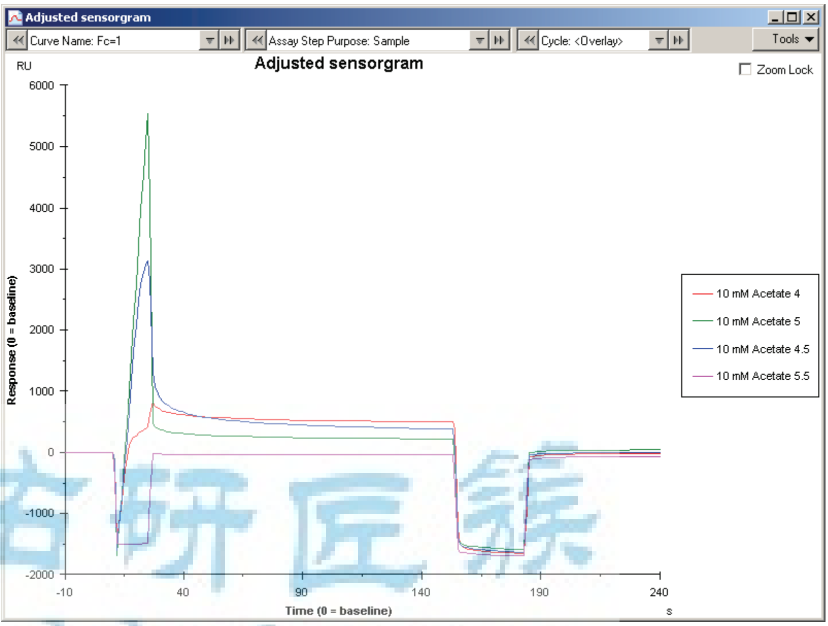
See Section 4.2.5. Immobilization pH scouting requires one position for ligand solution at each pH tested and one for the surface wash solution. Accept or change the rack positions for the various solutions required (see Section 4.2.5), then save the wizard template and start the run.

5. *Prepare run protocol*

Edit the **Prepare Run Protocol** text if desired (Section 4.2.6). This text will be displayed at the start of a run when the wizard template is used.

pH scouting results

When the wizard run is completed, the results are opened automatically in the Evaluation Software, with an overlay plot of the sensorgrams adjusted to the start of the sample injection.

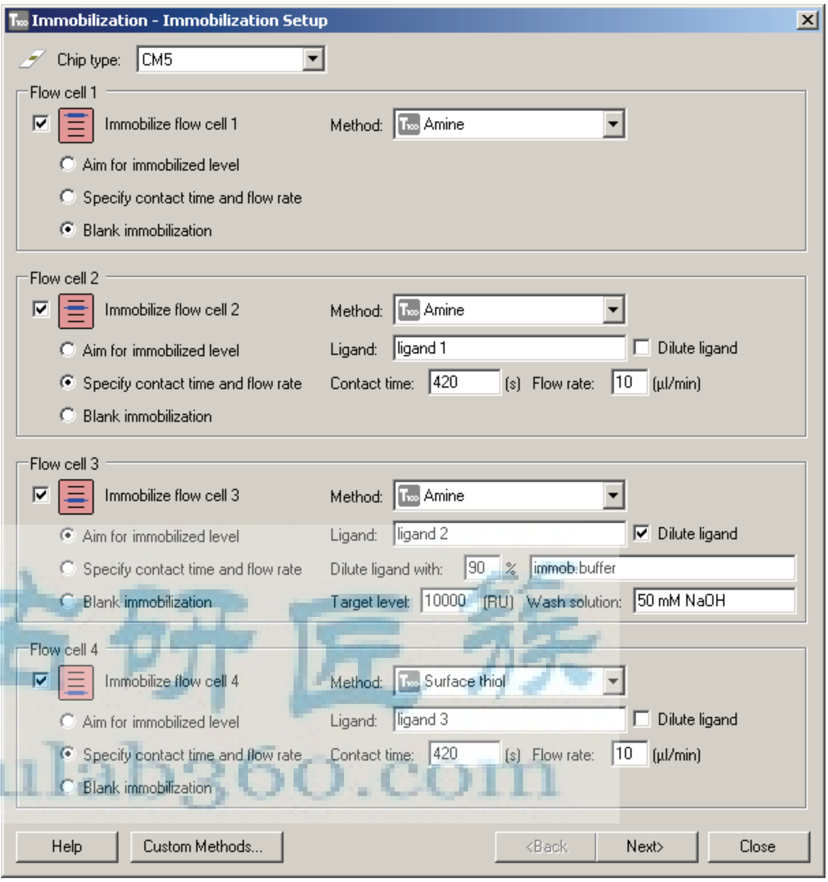


Choose the optimum buffer pH on the basis of the binding behaviour: at pH suitable for immobilization, the ligand binds rapidly to the surface during the injection and dissociates completely after the end of the injection. The optimum pH is generally the highest value that gives sufficient ligand binding (not necessarily the value that gives the highest ligand binding). Beware of conditions that give irregular sensorgrams with incomplete dissociation: this behavior often indicates aggregation or denaturation of the ligand.

4.3.2 Immobilization

The immobilization wizard supports immobilization of ligand in any combination of the four flow cells in one run. Immobilization in each flow cell is performed independently in a separate cycle, so that different ligands and/or immobilization conditions can be used in the different flow cells. See the Biacore Sensor Surface Handbook for more information about ligand immobilization.

1. *Immobilization setup*



Immobilization - Immobilization Setup

Chip type: CM5

Flow cell 1
☑ Immobilize flow cell 1 Method: Amine
○ Aim for immobilized level
○ Specify contact time and flow rate
● Blank immobilization

Flow cell 2
☑ Immobilize flow cell 2 Method: Amine
○ Aim for immobilized level Ligand: ligand 1 ☐ Dilute ligand
● Specify contact time and flow rate Contact time: 420 (s) Flow rate: 10 (µl/min)
○ Blank immobilization

Flow cell 3
☑ Immobilize flow cell 3 Method: Amine
● Aim for immobilized level Ligand: ligand 2 ☑ Dilute ligand
○ Specify contact time and flow rate Dilute ligand with: 90 % immob.buffer
○ Blank immobilization Target level: 10000 (RU) Wash solution: 50 mM NaOH

Flow cell 4
☑ Immobilize flow cell 4 Method: Surface thiol
○ Aim for immobilized level Ligand: ligand 3 ☐ Dilute ligand
● Specify contact time and flow rate Contact time: 420 (s) Flow rate: 10 (µl/min)
○ Blank immobilization

The choice of **Chip type** determines the predefined methods that are available for immobilization. The type chosen when the chip was docked is chosen by default: if you change the chip type you will be able to create and save an immobilization wizard template, but you must dock a corresponding chip type before the immobilization can be performed.

Check the flow cells where you want to perform immobilization. For each flow cell, set the 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 below). Predefined methods are marked with a T100 icon (T100) in the selection lists.

Choose the way in which immobilization will be controlled:

- If you choose **Aim for immobilized level**, you specify a target level. The immobilization procedure will attempt to reach this level as described below.

- If you choose **Specify contact time and flow rate**, enter the settings in the respective fields.

- If you choose **Blank immobilization**, the surface will be activated and deactivated in accordance with the immobilization method but no ligand will be injected.

Enter the ligand name. To dilute the ligand solution immediately before injection, check **Dilute ligand** and enter a percentage value and a solution name. This option can be used for ligands that have limited stability in immobilization buffer, and that are diluted from a stock solution just before immobilization. A setting of 90% will mix one part of ligand solution with 9 parts of the specified diluent.

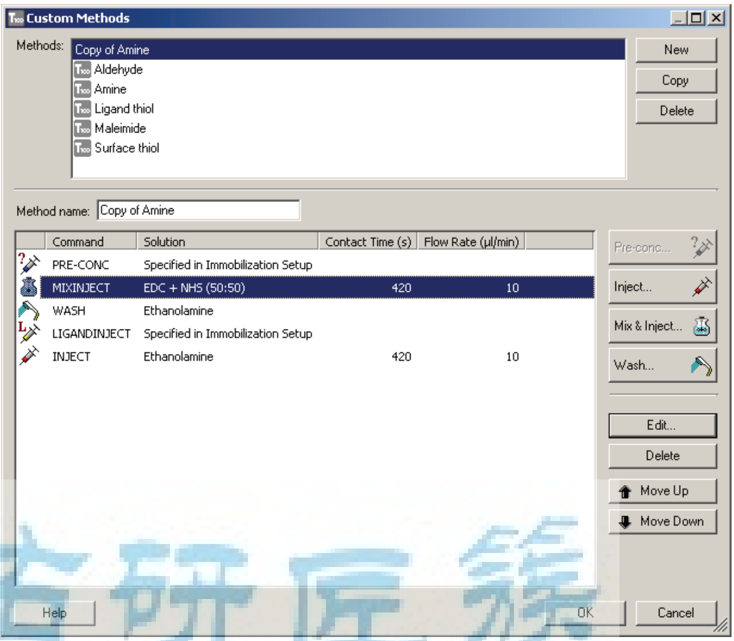
Aiming for immobilized level

The option **Aim for immobilized level** injects a pulse of ligand over the unactivated surface in order to estimate the rate of 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 injection injects 10 µl ligand solution at a flow rate of 5 µl/min, giving a contact time of 2 minutes. This injection 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 option can be used to conserve valuable ligand without losing the benefits of aiming for a target immobilization level. This option is particularly useful for sensor chips where preconcentration cannot be performed, e.g. Sensor Chip SA.

Custom methods

Click **Custom Methods** to define customized immobilization methods.



Methods:
- Copy of Amine
- Aldehyde
- Amine
- Ligand thiol
- Maleimide
- Surface thiol

Method name: Copy of Amine

Command	Solution	Contact Time (s)	Flow Rate (µl/min)
PRE-CONC	Specified in Immobilization Setup		
MIXINJECT	EDC + NHS (50:50)	420	10
WASH	Ethanolamine		
LIGANDINJECT	Specified in Immobilization Setup		
INJECT	Ethanolamine	420	10

Click **New** to create a new blank method. Select an existing method and click **Copy** or **Delete** to make a copy of the method or delete it respectively. You cannot delete the predefined methods (marked with a T100 icon).

For a new method, enter a name in the **Method name** field. Construct the sequence of injections for the immobilization 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:

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	performs an injection of a specified solution with a specified contact time and flow rate. Values 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 (but not the sensor surface). The wash solution is specified 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 wizard template: if you need the same or slightly modified method in a different template, save a copy of the template and then edit the method.

2. System preparations
Check the **System preparations** options as required (see Section 4.2.4).

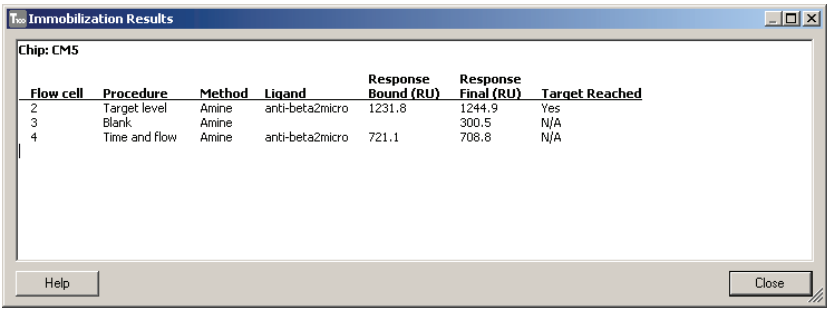
3. Rack positions
Accept or change the rack positions for the various solutions required (see Section 4.2.5), then save the wizard template and start the run.

4. Prepare run protocol
Edit the **Prepare Run Protocol** text if desired (Section 4.2.6). This text will be displayed at the start of a run when the wizard template is used.

Immobilization results

The results of an immobilization run are summarized in the Control Software and logged in the **Chip Properties** (see Section 2.3.4) when the run is completed.

Note: This result presentation is not shown if the run is opened in the Evaluation Software.

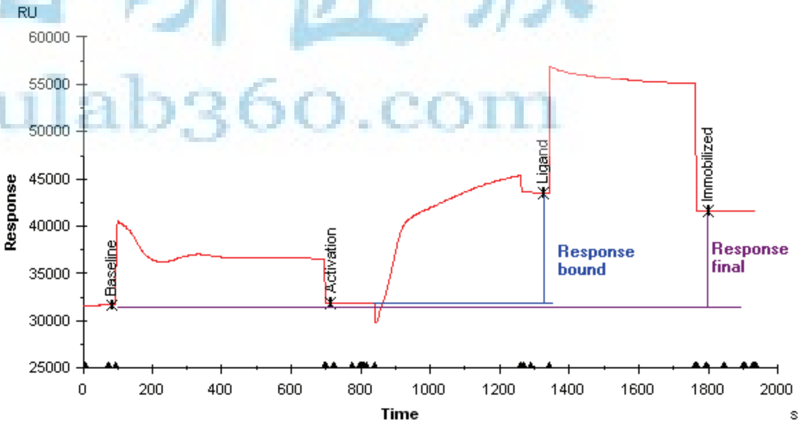


Chip: CM5

Flow cell	Procedure	Method	Ligand	Response Bound (RU)	Response Final (RU)	Target Reached
2	Target level	Amine	anti-beta2micro	1231.8	1244.9	Yes
3	Blank	Amine			300.5	N/A
4	Time and flow	Amine	anti-beta2micro	721.1	708.8	N/A

The summary lists the procedure and method, the name of the ligand and whether the target was reached with **Aim for immobilized level**. Two response values are reported, 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 that the **Response bound** value does not include the contribution from activation by EDC/NHS. For low ligand levels, this value usually gives a better indication of the amount of ligand immobilized.



Result files from immobilization can also be opened in the Evaluation Software if you want to prepare other sensorgram displays or plots (see Chapter 7).

4.4 Assay development wizards

4.4.1 Regeneration scouting

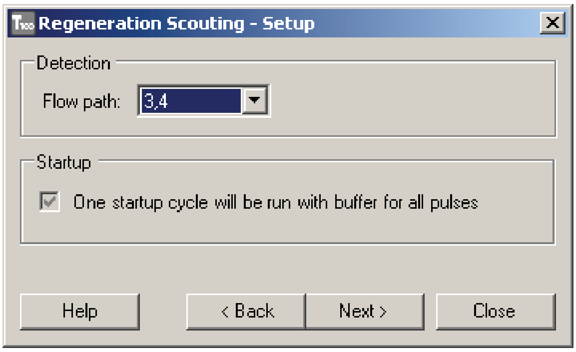
The regeneration scouting wizard guides you through the process of finding suitable regeneration conditions for your sensor surface. The principles of regeneration scouting are described in the Biacore Sensor Surface Handbook. Briefly, regeneration scouting is performed by testing repeated cycles of analyte injection and regeneration over a range of regeneration conditions, and assessing the results on the basis of trends in analyte response and baseline levels. The analyte concentration should be relatively high for best results. The analyte response reflects the binding capacity (ligand activity) of the surface, while the baseline level indicates the extent of regeneration. Each condition should be tested for at least 3 cycles in sequence (recommended number 5) in order to detect trends in the regeneration behaviour with the given condition. When testing multiple conditions, start with the mildest conditions to minimize the risk of losing ligand activity at the beginning of the scouting series.

1. Injection sequence

Choose the injection sequence for the regeneration scouting (see Section 4.2.1). One sample injection and one or two regeneration injections are required. Most sensor surfaces can be adequately regenerated with a single injection, but some situations may benefit from using multiple injections.

2. Setup

Specify the flow path.


Regeneration Scouting - Setup

Detection

Flow path: 3,4

Startup

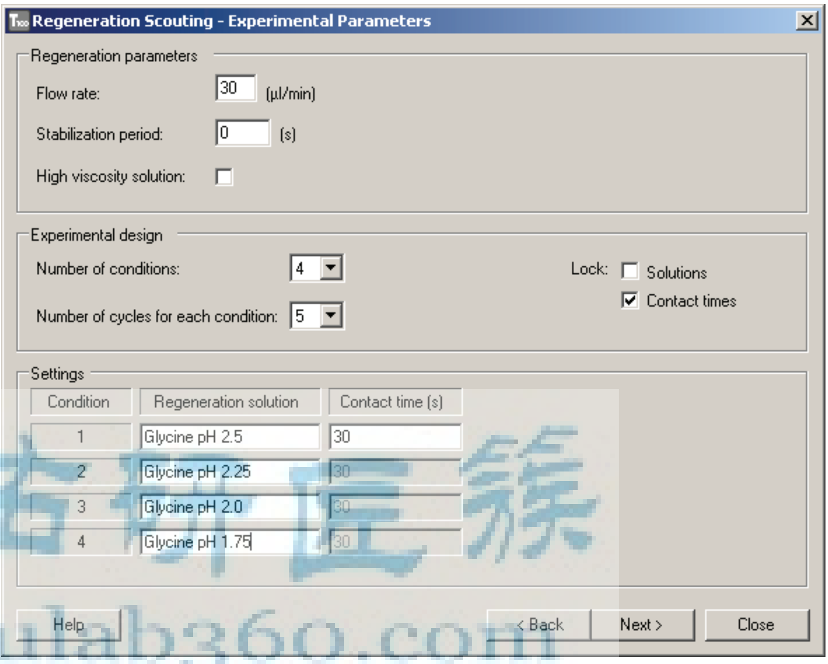
☑ One startup cycle will be run with buffer for all pulses

Regeneration scouting always starts with one startup cycle with the same injection sequence as the scouting cycles but with injection of buffer for all injections. Note that this differs from the startup cycle construction in the other application wizards.

3. *Injection parameters*

Specify the injection parameters for each injection in the cycle (see Section 4.2.3). The same sample will be used for all cycles.

4. *Experimental parameters*



Regeneration Scouting - Experimental Parameters

Regeneration parameters

Flow rate: 30 (µl/min)

Stabilization period: 0 (s)

High viscosity solution: ☐

Experimental design

Number of conditions: 4

Number of cycles for each condition: 5

Lock: ☐ Solutions ☑ Contact times

Settings

Condition	Regeneration solution	Contact time (s)
1	Glycine pH 2.5	30
2	Glycine pH 2.25	30
3	Glycine pH 2.0	30
4	Glycine pH 1.75	30

This dialog box determines the design of your regeneration scouting. Set the number of conditions to test and the number of cycles for each condition and specify the conditions in the **Settings** frame. The default number of cycles for each condition is five. You may use fewer cycles to shorten the total run time for exploratory work, but five cycles are recommended for fine-tuning conditions in order to reveal trends in the regeneration performance.

Use variants of the same kind of regeneration conditions (e.g. different pH values or different concentrations of ethylene glycol) within the same run. Results are most easily interpreted if you use a new flow cell or sensor chip for each kind of regeneration condition that you test, so that the outcome with one kind of condition is not affected by the history of exposing the ligand to another.

You may choose to lock the solutions or the contact time used for regeneration tests, so that all conditions will use the same setting for the locked parameter. Do not vary both the solution and the contact time at the same time: the results may be difficult to interpret clearly.

Check **High viscosity solution(s)** if any of the regeneration solutions tested has a relative viscosity higher than about 3 (corresponding to about 35% glycerol or 40% ethylene glycol at 20°C). This will modify the injection procedure for better handling of viscous solutions.

5. *System preparations*

Check the **System preparations** options as required (see Section 4.2.4).

6. *Rack positions*

Accept or change the rack positions for the various solutions required (see Section 4.2.5), then save the wizard template and start the run.

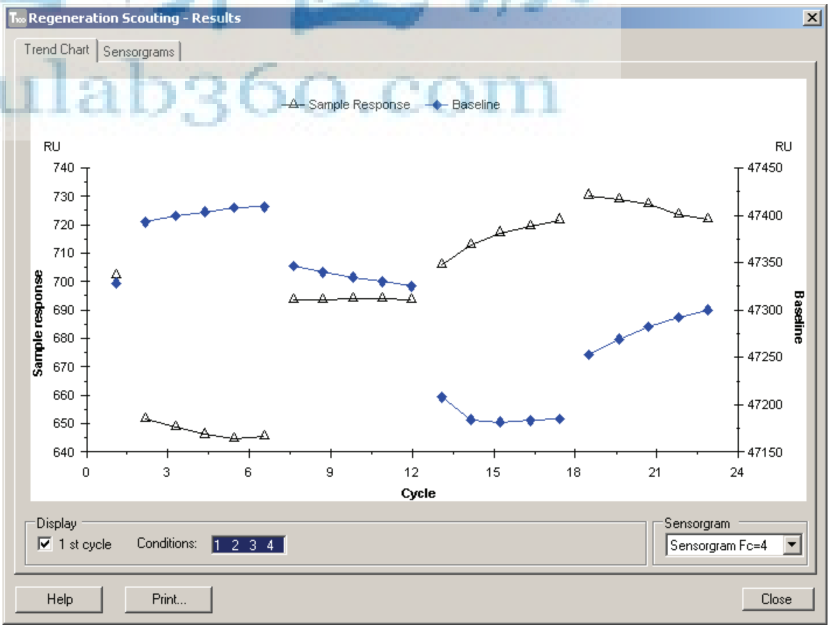
7. *Prepare run protocol*

Edit the **Prepare Run Protocol** text if desired (Section 4.2.6). This text will be displayed at the start of a run when the wizard template is used.

Regeneration scouting results

Regneration scouting results are presented in the Control Software when the run is completed.

Note: This result presentation is not shown if the run is opened in the Evaluation Software.



☑ 1 st cycle

Conditions: 1 2 3 4

Sensorgram: Sensorgram Fc=4

The **Trend chart** tab shows the results as a plot of baseline and sample response for each cycle in the run, grouped by regeneration conditions. Conditions are identified in tool tips for the data points (place the cursor on a point for a couple of seconds to display the tool tip).

Note: Report points are set before the sample injection for baseline and shortly after the sample injection for sample response. Thus the points for the first cycle indicate the starting values, while those for subsequent cycles each indicate the effect of the previous cycle.

Check **1st cycle** to include the starting values derived from the first sample cycle in the plot. (This cycle is shown as cycle number 2: cycle 1 is the startup cycle that is not shown in the plot.)

Select which conditions to display in the **Conditions** box. Use Shift-click to make multiple adjacent selections, Ctrl-click to make multiple non-adjacent selections. The scale of the display will be adjusted according to the number of cycles displayed.

Select which curves to display in the **Sensorgram** box.

The **Sensorgrams** tab shows the sensorgrams for regeneration scouting. Select the conditions and cycles to display in the respective boxes. Check **Zoom lock** to keep the scale fixed when the choice of sensorgrams is changed.

Result files from regeneration scouting can also be opened in the Evaluation Software if you want to prepare other sensorgram displays or plots (see Chapter 7).

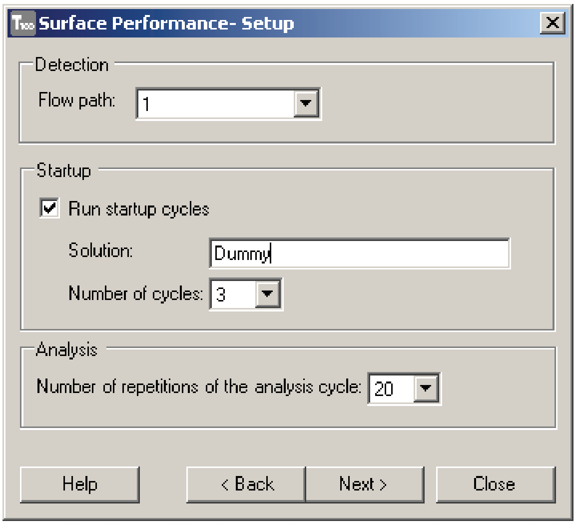
4.4.2 Surface performance

The surface performance wizard allows you to test the robustness of your surface by performing repetitions of the same analysis cycle. The number of repetitions is in practice limited by the capacity of the sample plate (the software allows up to 400 cycles). The cycle requires one sample injection, and can also include capture and enhancement steps and one or two regeneration steps. Use this wizard for example when you want to confirm that the regeneration conditions that you identified in regeneration scouting hold good for an extended number of cycles.

1. *Injection sequence*

Choose the injection sequence for the surface performance test cycle (see Section 4.2.1).

2. *Assay setup*



Flow path: 1

☑ Run startup cycles

Solution: Dummy

Number of cycles: 3

Number of repetitions of the analysis cycle: 20

Specify the flow path and start-up cycles (see Section 4.2.2).

Set the number of repetitions of the analysis cycle according to the purpose of the surface performance test. As a general guide, the test should run for at least as many cycles as will be used normally in the assay.

3. *Injection parameters*
Specify the injection parameters for each injection in the cycle (see Section 4.2.3).

4. *System preparations*
Check the **System preparations** options as required (see Section 4.2.4).

5. *Rack positions*
Accept or change the rack positions for the various solutions required (see Section 4.2.5), then save the wizard template and start the run.

6. *Prepare run protocol*
Edit the **Prepare Run Protocol** text if desired (Section 4.2.6). This text will be displayed at the start of a run when the wizard template is used.

Surface performance results

When the wizard run is completed, the results are opened automatically in the Evaluation Software. Examine the plots of baseline and sample response against cycle number. The response values should ideally be unchanged throughout the run.

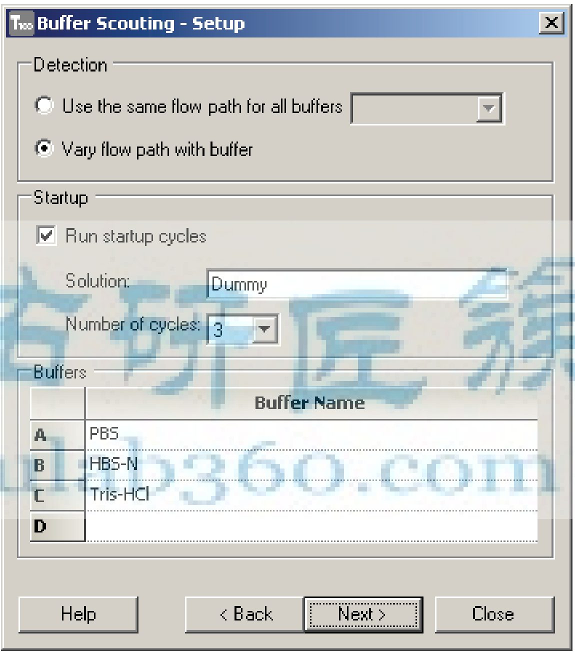
4.4.3 Buffer scouting

The buffer scouting wizard helps you to test the effect of up to four different buffers on your assay, using the buffer selector valve to switch running buffers.

1. *Injection sequence*

Choose the injection sequence for the buffer scouting (see Section 4.2.1).

2. *Assay setup*



Detection

○ Use the same flow path for all buffers

● Vary flow path with buffer

Startup

☑ Run startup cycles

Solution: Dummy

Number of cycles: 3

Buffers

	Buffer Name
A	PBS
B	HBS-N
C	Tris-HCl
D	

Specify the flow path and start-up cycles. The flow path can either be chosen explicitly (in which case the same flow path will be used for each buffer), or set to vary with the buffer (in which case a single flow cell will be used for each buffer, with flow cells 1, 2, 3 and 4 for buffers A, B, C and D respectively). If start-up cycles are chosen, separate rack positions will be created for the start-up sample solution in each buffer tested, and the start-up cycles will be run at the start of each buffer test.

Specify the buffers you want to test. You can enter up to four different buffers. The buffers will be tested in the order given.

3. Injection parameters

Specify the injection parameters for each injection in the cycle (see Section 4.2.3).

Ligands for capture, samples and enhancement reagents should be prepared in each of the running buffers tested. Separate rack positions will be created for samples in each buffer (for example, running buffer scouting with 4 buffers and 5 samples will require 20 sample positions).

4. Sample parameters

Enter the samples to be tested in the buffer scouting. The scouting procedure will work through the sample table for the first buffer before switching to the next buffer.

5. System preparations

Check the **System preparations** options as required (see Section 4.2.4).

6. Rack positions

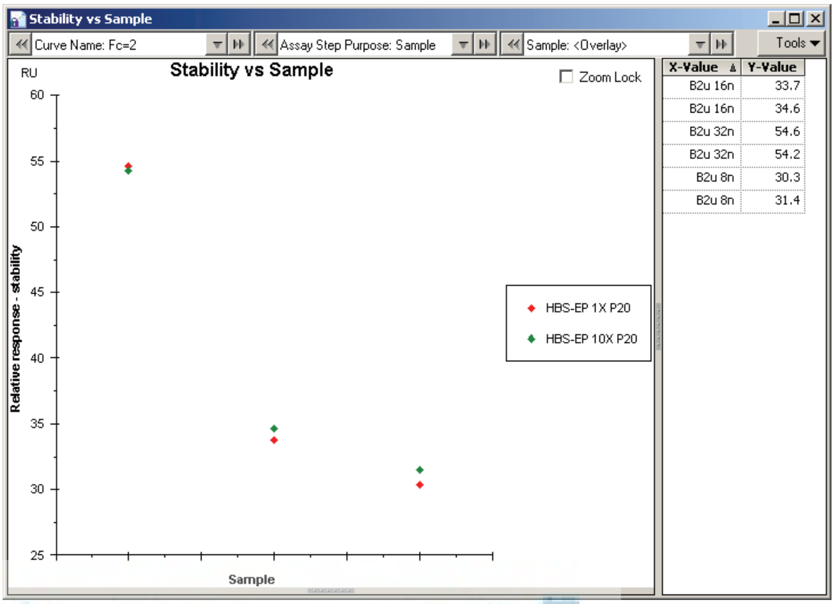
Accept or change the rack positions for the various solutions required (see Section 4.2.5), then save the wizard template and start the run.

7. Prepare run protocol

Edit the **Prepare Run Protocol** text if desired (Section 4.2.6). This text will be displayed at the start of a run when the wizard template is used.

Buffer scouting results

When the wizard run is completed, the results are opened automatically in the Evaluation Software. In addition to the general predefined plots (Section 6.4), plots of binding and stability against sample are created to visualize the behavior in the different buffers.



X-Value	Y-Value
B2u 16n	33.7
B2u 16n	34.6
B2u 32n	54.6
B2u 32n	54.2
B2u 8n	30.3
B2u 8n	31.4

4.5 Assay wizards

4.5.1 Binding analysis

The binding analysis wizard supports injection of up to four samples in series, in addition to ligand capture, enhancement and regeneration steps. This wizard is suitable for analysis of applications like multi-component complex formation and pair-wise epitope mapping, as well as simple applications like screening for binding partners to an immobilized ligand.

1. Injection sequence

Choose the injection sequence for the binding analysis (see Section 4.2.1). Up to four sequential sample injections may be included in each cycle.

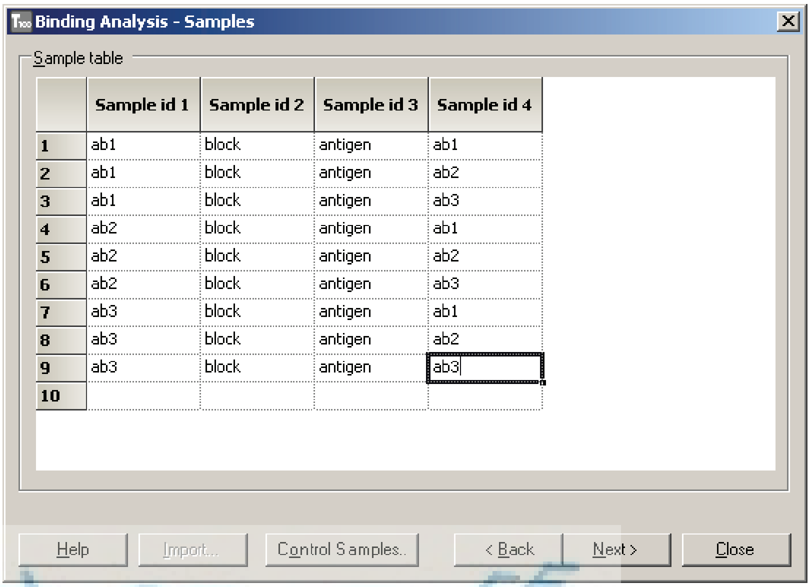
2. Setup

Specify the flow path and start-up cycles (see Section 4.2.2).

3. Injection parameters

Specify the injection parameters for each injection in the cycle (see Section 4.2.3). One sample injection panel will be created for each sample injection in the injection sequence.

4. *Sample parameters*

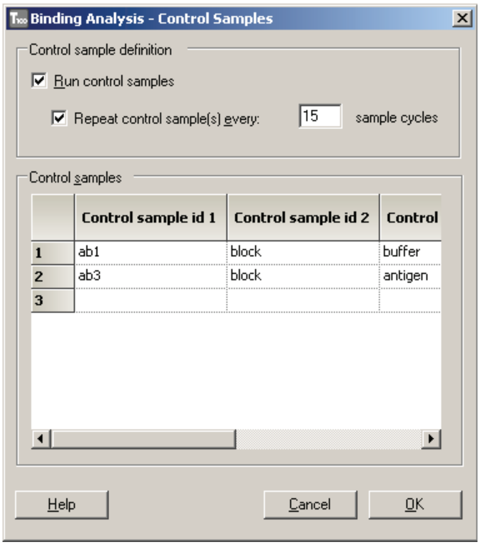


	Sample id 1	Sample id 2	Sample id 3	Sample id 4
1	ab1	block	antigen	ab1
2	ab1	block	antigen	ab2
3	ab1	block	antigen	ab3
4	ab2	block	antigen	ab1
5	ab2	block	antigen	ab2
6	ab2	block	antigen	ab3
7	ab3	block	antigen	ab1
8	ab3	block	antigen	ab2
9	ab3	block	antigen	ab3
10				

The sample table contains one column for each sample injection in the injection sequence. New rows are created as you enter data in the table. (The illustration above shows how the wizard could be used to set up a pair-wise epitope mapping experiment.)

Click **Import** to import the sample data from an external file. Import of sample information must be enabled in **Tools:Preferences** to use this function. See Appendix A for details of import functions and file formats.

Click **Control Samples** to enter control samples for the run.



☑ Run control samples

☑ Repeat control sample(s) every: 15 sample cycles

	Control sample id 1	Control sample id 2	Control
1	ab1	block	buffer
2	ab3	block	antigen
3			

Specify the details of control samples and the frequency with which they should be run. Check the **Repeat control samples every...** box and enter a number of cycles to repeat the control samples at intervals during the run. The controls will then be run at the beginning of the assay and at the specified interval. If this box is not checked, the control samples will be run once at the beginning of the assay. You can uncheck the **Run control samples** box to skip control samples without deleting the details from the method.

5. *System preparations*

Check the **System preparations** options as required (see Section 4.2.4).

6. *Rack positions*

Accept or change the rack positions for the various solutions required (see Section 4.2.5), then save the wizard template and start the run.

7. *Prepare run protocol*

Edit the **Prepare Run Protocol** text if desired (Section 4.2.6). This text will be displayed at the start of a run when the wizard template is used.

Binding analysis results

When the wizard run is completed, the results are opened automatically in the Evaluation Software. Predefined plots (Section 6.4) are created for each sample.

4.5.2 Concentration analysis

The concentration analysis wizard helps you to set up an assay for determining analyte concentration in samples with the help of a calibration curve using known concentrations. Control samples may be included at intervals to monitor the stability of the assay.

1. *Injection sequence*

Choose the injection sequence for the concentration analysis (see Section 4.2.1).

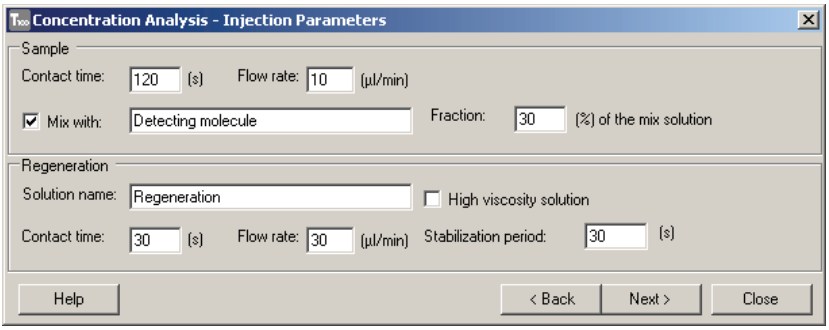
2. *Assay setup*

Specify the flow path and start-up cycles (see Section 4.2.2). Start-up cycles are recommended for concentration assays to ensure that the initial response drift that may occur with a new chip does not interfere with the first measurements.

3. *Injection parameters*

Specify the injection parameters for each injection in the cycle (see Section 4.2.3).

The sample injection for concentration analysis can be extended to include a mixing function, whereby sample is mixed with a specified proportion of a second fixed solution:



Concentration Analysis - Injection Parameters

Sample

Contact time: 120 (s) Flow rate: 10 (µl/min)

☑ Mix with: Detecting molecule Fraction: 30 (%) of the mix solution

Regeneration

Solution name: Regeneration ☐ High viscosity solution

Contact time: 30 (s) Flow rate: 30 (µl/min) Stabilization period: 30 (s)

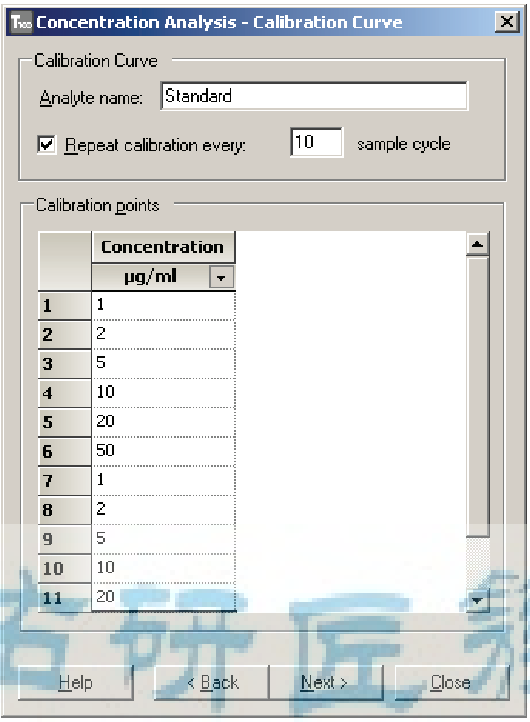
This feature enables inhibition assay formats where samples are mixed with a constant proportion of a detecting molecule solution. The value specified for **Fraction** refers to the proportion of the fixed component in the final mixture: for example, a value of 30% will mix 7 parts of sample with 3 parts of the specified solution.

The volumes of sample and mixing solution used are determined automatically so that the final volume of mixed solution is sufficient for the injection.

Notes: The mixing function is not supported in 384-well microplates. The wells on these plates are too small for reliable mixing with the autosampler needle.

Mixing in the autosampler is very reproducible, but high accuracy cannot be guaranteed. If your application requires accurate mixing proportions, mix the samples outside the autosampler.

4. *Calibration curve*



Concentration Analysis - Calibration Curve

Calibration Curve

Analyte name: Standard

☑ Repeat calibration every: 10 sample cycle

Calibration points

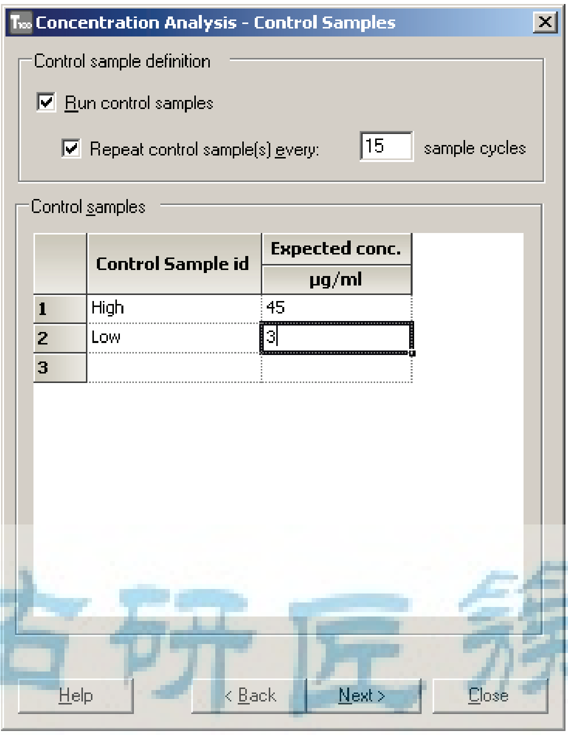
	Concentration µg/ml
1	1
2	2
3	5
4	10
5	20
6	50
7	1
8	2
9	5
10	10
11	20

Specify the details of the calibration curve for the concentration measurement.

Check **Repeat calibration** and enter a number of sample cycles to repeat the calibration curve at intervals during the assay. The calibration curve will be run at the beginning of the assay and at the specified interval. Thus specifying a repeat every 15 sample cycles and running 35 samples will result in calibration curves at the beginning and after samples 15 and 30. If the **Repeat calibration** box is not checked, calibration will be run once at the beginning of the assay.

Enter the concentrations for the calibration points on the curve. You must enter at least two concentrations for the calibration curve. (Two concentrations are sufficient for a linear calibration curve, but if you intend to use the recommended four-parameter fitting function for the calibration curve, you need at least four points.) To run replicate concentrations, enter the same concentration on multiple rows. Calibration points will be run in the order entered. You can choose a different concentration unit if required from the pull-down list in the table header.

5. *Control samples*



Concentration Analysis - Control Samples

Control sample definition

☑ Run control samples

☑ Repeat control sample(s) every: 15 sample cycles

Control samples

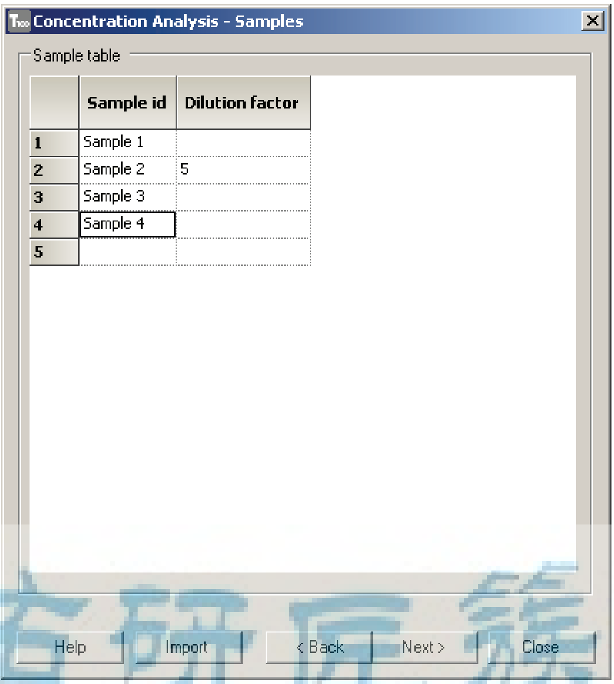
	Control Sample id	Expected conc. µg/ml
1	High	45
2	Low	3
3		

Specify the details of control samples and the frequency with which they should be run. You may choose not to run control samples at all: however, including control samples is generally recommended as an aid in assessing the performance of the assay.

Check the **Repeat control samples every...** box and enter a number of cycles to repeat the control samples at intervals during the run. The controls will then be run at the beginning of the assay and at the specified interval. If this box is not checked, the control samples will be run once at the beginning of the assay. Since control samples are presented in the evaluation software as a trend plot (see Section 8.2.2), running repeated controls throughout the assay is recommended.

Control samples are specified in terms of sample ID and expected concentration. The expected concentrations should lie within the range covered by the calibration curve.

6. *Samples*



	Sample id	Dilution factor
1	Sample 1	
2	Sample 2	5
3	Sample 3	
4	Sample 4	
5		

Enter the details of the samples to be analyzed. Each sample is defined by a sample ID and a dilution factor: the dilution factor is used during evaluation to calculate the measured concentration in the original undiluted sample. For undiluted samples (dilution factor 1), the **Dilution factor** column may be left blank.

Samples will be analyzed in the order entered. To analyze replicate samples, enter same sample on multiple rows.

Click **Import** to import the sample data from an external file. Import of sample information must be enabled in **Tools:Preferences** to use this function. See Appendix A for details of import functions and file formats.

7. *System preparations*

Check the **System preparations** options as required (see Section 4.2.4).

8. *Rack positions*

Accept or change the rack positions for the various solutions required (see Section 4.2.5), then save the wizard template and start the run.

9. *Prepare run protocol*

Edit the **Prepare Run Protocol** text if desired (Section 4.2.6). This text will be displayed at the start of a run when the wizard template is used.

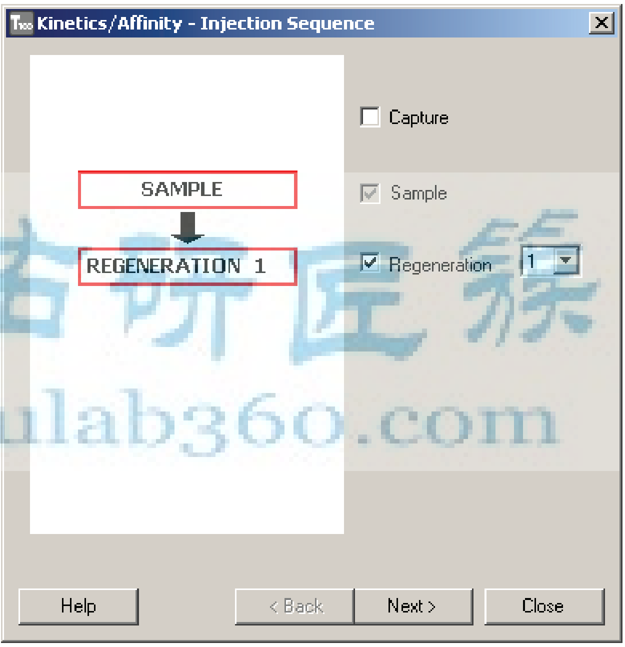
Evaluation

Chapter 8 describes how to evaluate concentration assays.

4.5.3 Kinetics/Affinity

The Kinetics/Affinity wizard guides you through the setup of experiments to determine kinetic constants or affinity constants for an interaction. Wizards for control experiments relevant to kinetic analysis are described in Section 4.6.

1. *Injection sequence*



☐ Capture

☑ Sample

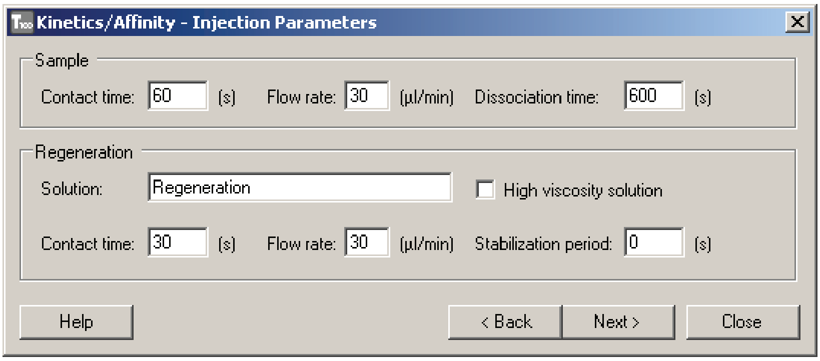
☑ Regeneration 1

Choose the injection sequence for the assay (see Section 4.2.1). The Kinetics wizard supports capture but not enhancement injections. The same injection sequence will be used for all selected assay steps.

2. *Assay setup*

Specify the flow path and start-up cycles (see Section 4.2.2). Only reference subtracted detection using either Fc2-1 or Fc4-3 is available for kinetic analysis. Start-up cycles are recommended for kinetics experiments to ensure that the initial response drift that may occur with a new chip does not interfere with the first measurements.

3. *Injection parameters*


Kinetics/Affinity - Injection Parameters

Sample

Contact time: 60 (s) Flow rate: 30 (µl/min) Dissociation time: 600 (s)

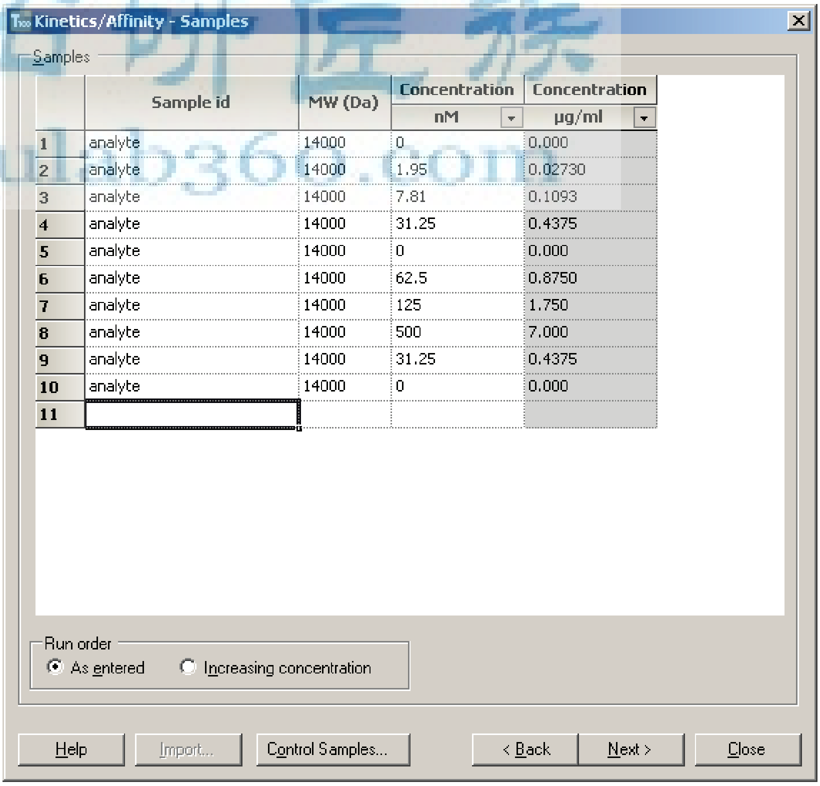
Regeneration

Solution: Regeneration ☐ High viscosity solution

Contact time: 30 (s) Flow rate: 30 (µl/min) Stabilization period: 0 (s)

Specify the injection parameters for each injection in the cycle (see Section 4.2.3). The sample injection for kinetics measurement has an additional setting for dissociation time. This is the time for which dissociation will be monitored after the end of the injection without disturbances from flow system washing procedures.

4. *Sample parameters*


Kinetics/Affinity - Samples

Samples

	Sample id	MW (Da)	Concentration nM	Concentration µg/ml
1	analyte	14000	0	0.000
2	analyte	14000	1.95	0.02730
3	analyte	14000	7.81	0.1093
4	analyte	14000	31.25	0.4375
5	analyte	14000	0	0.000
6	analyte	14000	62.5	0.8750
7	analyte	14000	125	1.750
8	analyte	14000	500	7.000
9	analyte	14000	31.25	0.4375
10	analyte	14000	0	0.000
11				

Run order

● As entered ○ Increasing concentration

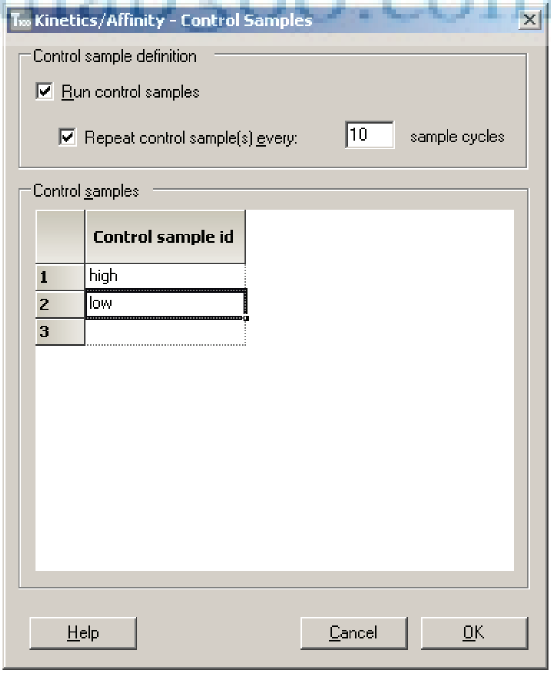
Enter the details of the samples for kinetic or affinity determination. For each analyte, a zero concentration sample and at least five non-zero concentrations, one of which is run in duplicate, are recommended. Concentrations are entered in the left-hand **Concentration** column, either in molar or weight-based units. Choose the concentration units from the pull-down list in the column header. If a weight-based concentration unit is chosen (e.g. µg/ml) a molecular weight must also be specified. When a molecular weight is entered, the right-hand **Concentration** column displays the conversion from molar to weight-based or *vice versa*. Samples with the same sample name may not be given different molecular weights.

The samples may be analysed either in the order entered in the table or sorted in increasing concentration. The order displayed in the sample table is not affected by the choice of run order.

If you enter samples with different names, they will be handled as separate concentration series regardless of the order in which they are entered. The samples will be run as separate concentration series even if the order is mixed in this dialog: thus samples entered in the order A, B, A, A, B, A, B, B… will be run in the order A, A, A, A, B, B, B, B… The **Run order** setting applies within each concentration series.

Click **Import** to import the sample data from an external file. Import of sample information must be enabled in **Tools:Preferences** to use this function. See Appendix A for details of import functions and file formats.

Click **Control Samples** to enter control samples for the run.



Kinetics/Affinity - Control Samples

Control sample definition

☑ Run control samples

☑ Repeat control sample(s) every: 10 sample cycles

Control samples

	Control sample id
1	high
2	low
3	

Specify the details of control samples and the frequency with which they should be run. Check the **Repeat control samples every…** box and enter a number of cycles to repeat the control samples at intervals during the run. The controls will then be run at the beginning of the assay and at the specified interval. If this box is not checked, the control samples will be run once at the beginning of the assay.

5. System preparations
Check the **System preparations** options as required (see Section 4.2.4).

6. Rack positions
Accept or change the rack positions for the various solutions required (see Section 4.2.5), then save the wizard template and start the run.

7. Prepare run protocol
Edit the **Prepare Run Protocol** text if desired (Section 4.2.6). This text will be displayed at the start of a run when the wizard template is used.

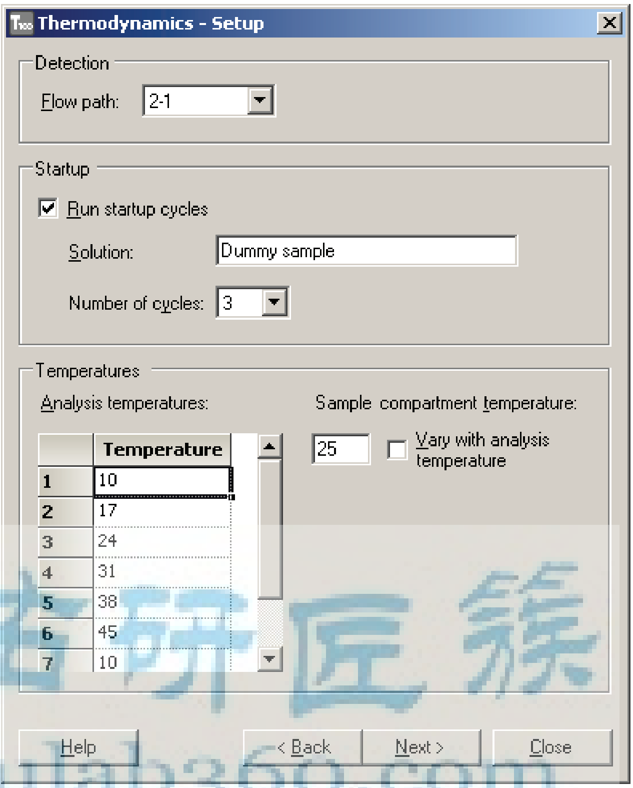
Evaluation
Chapter 9 describes how to evaluate kinetics assays.

4.5.4 Thermodynamics

The thermodynamics wizard supports kinetic and affinity determinations over a range of temperatures. The corresponding evaluation software extracts thermodynamic data from the dependence of rate and affinity constants on temperature (see Section 10.1).

1. Injection sequence
Choose the injection sequence for the assay (see Section 4.2.1). The thermodynamics wizard supports capture but not enhancement injections. The same injection sequence will be used for all selected assay steps.

2. *Assay setup*



Flow path: 2-1

☑ Run startup cycles

Solution: Dummy sample

Number of cycles: 3

Analysis temperatures:

	Temperature
1	10
2	17
3	24
4	31
5	38
6	45
7	10

Sample compartment temperature: 25 ☐ Vary with analysis temperature

Specify the flow path and start-up cycles (see Section 4.2.2). Start-up cycles are recommended for thermodynamics experiments to ensure that the initial response drift that may occur with a new chip does not interfere with the first measurements. Start-up cycles will be run at each temperature.

Enter the temperatures at which the measurements are to be performed. For most purposes, 5-7 temperatures will be adequate: fewer points make the determination of thermodynamic parameters uncertain, while more points increase the run time. Distribute the points evenly over the widest temperature interval that the ligand and analyte tolerate. Start from the lowest temperature to minimize the time needed for temperature equilibration between measurements (increasing the analysis temperature takes less time than decreasing it). The system will wait for a stable temperature between each determination. As an additional control, you may want to include a replicate of the first temperature at the end of the run.

Note: For robust determination of standard thermodynamic parameters (ΔG°, ΔH° and ΔS°), arrange the analysis temperatures so that 25°C is in the middle of the range (e.g. 10–40°). See Section 10.1 for further details.

Specify the sample compartment temperature, or check **Vary with analysis temperature** to change the sample compartment temperature automatically when the analysis temperature is changed.

3. *Injection parameters*

Specify the injection parameters for each injection in the cycle (see Section 4.2.3). The sample injection for thermodynamics measurement has an additional setting for dissociation time. This is the time for which dissociation will be monitored after the end of the injection without disturbances from flow system washing procedures.

4. *Sample parameters*

Enter the details of the samples. This dialog box is equivalent to the concentration series dialog for kinetics determination (see Section 4.5.3, step 4). Separate rack positions will be created for each sample at each temperature.

5. *System preparations*

Check the **System preparations** options as required (see Section 4.2.4). The settings for analysis temperature and sample compartment temperature cannot be changed here: the values for the first temperature as specified in step 2 are shown.

6. *Rack positions*

Accept or change the rack positions for the various solutions required (see Section 4.2.5), then save the wizard template and start the run.

7. *Prepare run protocol*

Edit the **Prepare Run Protocol** text if desired (Section 4.2.6). This text will be displayed at the start of a run when the wizard template is used.

Evaluation

Section 10.2 describes how to evaluate thermodynamic assays.

4.6 Control experiment wizards

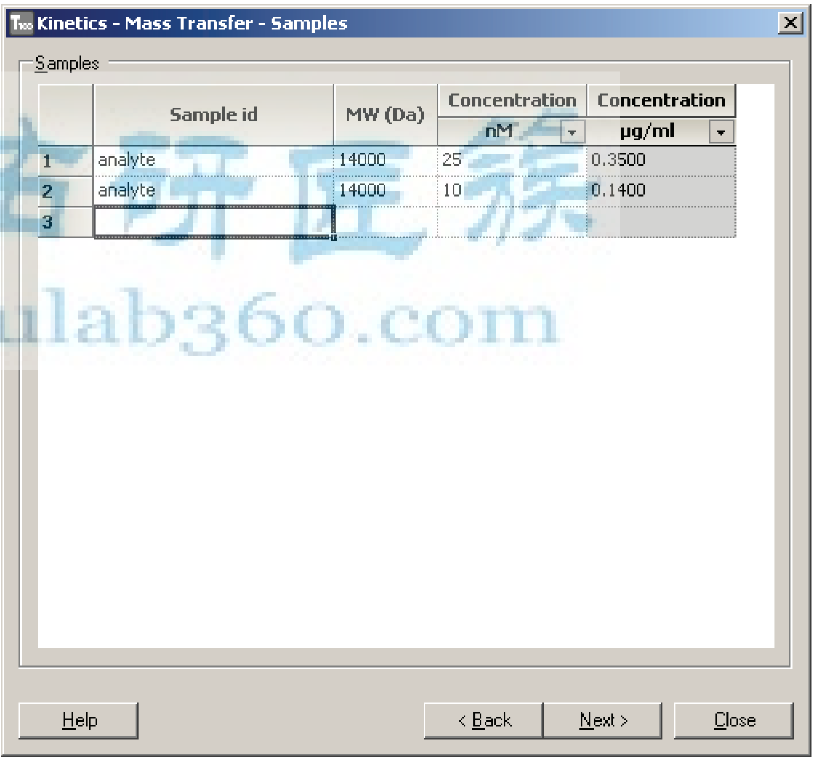
Two control experiments are currently supported by wizards, both for kinetic analyses:

- The **Mass transfer** control experiment analyses the interaction of one or more analyte concentrations at three different flow rates, to establish whether the observed binding rate varies with flow rate. A dependence of binding rate on flow rate indicates that the binding is limited to some extent by mass transfer of analyte to the sensor surface.

- The **Linked reactions** control experiment analyses the interaction of one or more analyte concentrations for different contact times, to identify a particular kind of deviation from a 1:1 binding mechanism. Variation of the dissociation behavior after the end of the injection with the contact time indicates that the observed binding consists of at least two processes, one where the analyte binds to the surface and a second where the surface-attached complex undergoes a stabilizing alteration such as a conformational change.

The control experiment wizards have the same structure as the kinetic analysis wizard, but differ in the details of the sample parameters step.

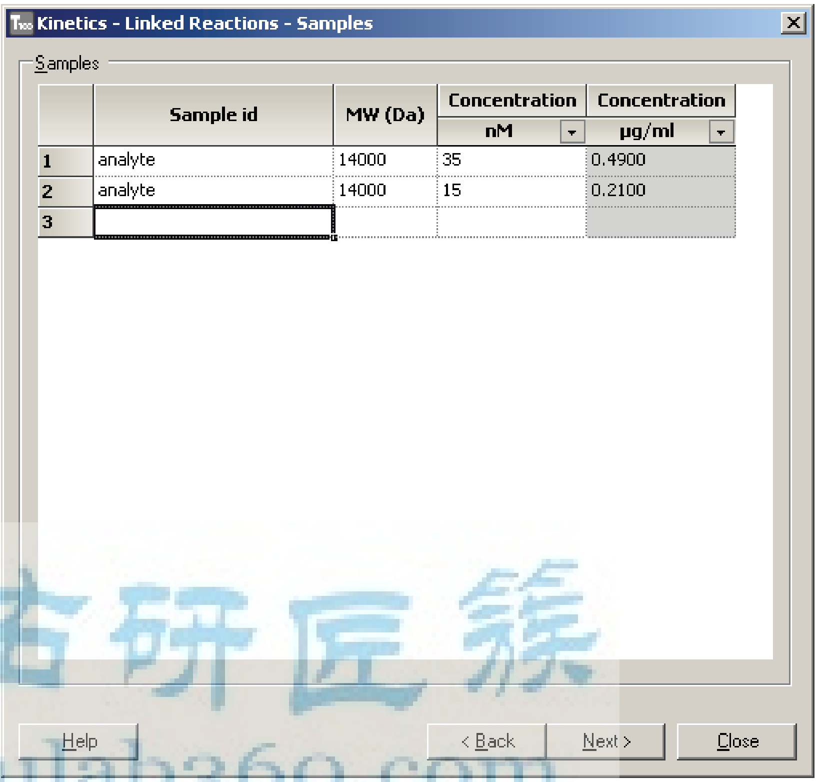
4.6.1 Mass transfer control



	Sample id	MW (Da)	Concentration nM	Concentration µg/ml
1	analyte	14000	25	0.3500
2	analyte	14000	10	0.1400
3				

Enter the details of the samples for the mass transfer control experiment. Each sample will be analyzed at fixed flow rates of 5, 15 and 75 µl/min, with a contact time of 1 minute and dissociation time of 2 minutes. Use an analyte concentration that gives readily measurable initial binding rates. Mass transfer limitation is not affected by analyte concentration, but dependence of binding rate on flow rate may be difficult to detect if the binding rate is too low or too high.

4.6.2 Linked reactions control



	Sample id	MW (Da)	Concentration nM	Concentration µg/ml
1	analyte	14000	35	0.4900
2	analyte	14000	15	0.2100
3				

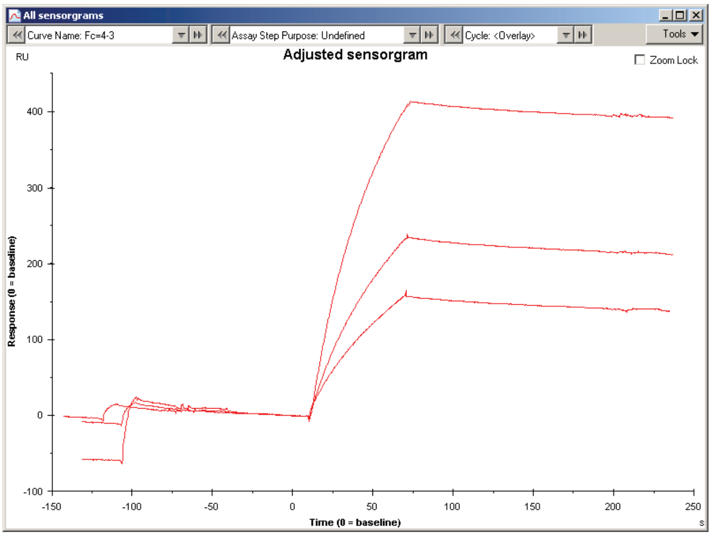
Enter the details of the samples for the linked reactions control experiment. Each sample will be analyzed at a flow rate of 10 µl/min with fixed contact times of 0.5, 3 and 10 minutes and a dissociation time of 10 minutes. Use one or more fairly high analyte concentrations, preferably so that steady state is approached or reached within the shortest contact time. The experiment is easiest to interpret if the interaction is maintained at steady state for varying lengths of time, so that the starting response for dissociation is constant.

4.6.3 Evaluation of control experiments

When the wizard run is completed, the results are opened automatically in the Evaluation Software, with overlay plot of adjusted sensorgrams according to the control experiment.

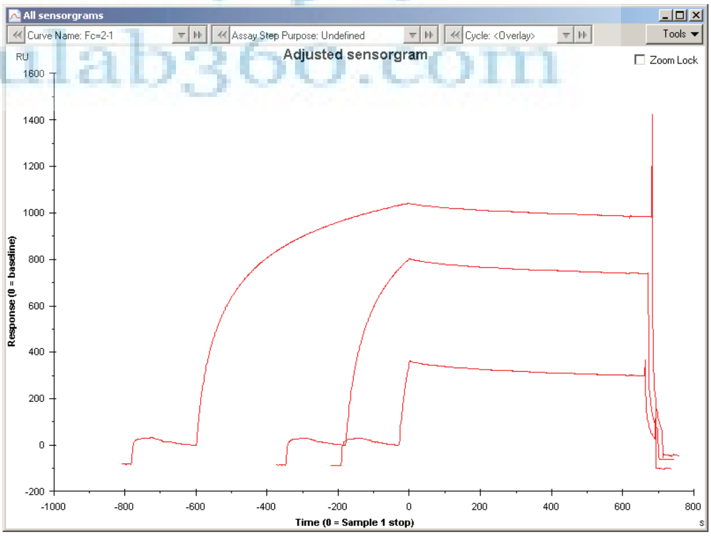
Mass transfer

The sensorgrams are adjusted to zero response and time at the baseline report point. Compare the observed binding rates at the different flow rates.



Linked reactions

The sensorgrams are adjusted to zero response at the baseline report point and to zero time at the end of the sample injection. Compare the observed dissociation rates at the different contact times.



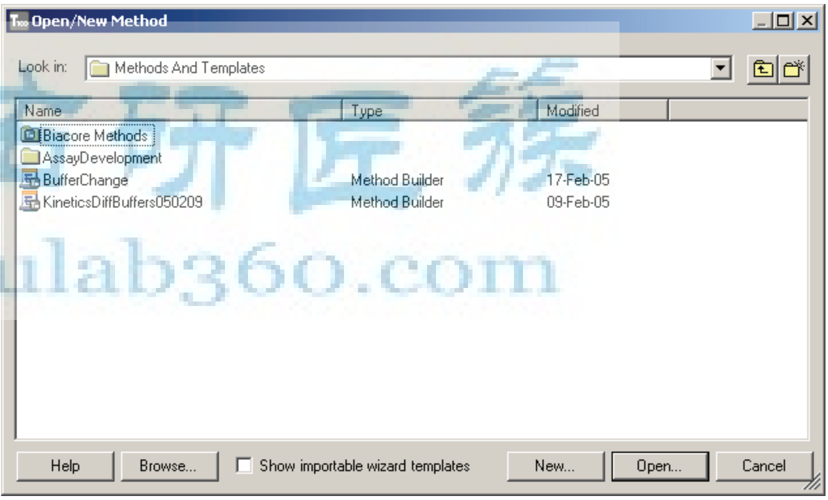
Note: If the binding does not reach a steady state in all sensorgrams (as in the example above) it may be easier to compare the dissociation rates if you use a second y-adjustment point at the end of the sample injection (see Section 7.1.3).

5. Methods

Methods in Biacore T100 offer flexibility in instrument control, providing support for applications that cannot conveniently be handled with wizards. Methods are constructed with the ***Method Builder*** tool as described in this chapter. Templates from application wizards can be opened in Method Builder (Section 5.1) to provide a starting point for development of customized applications. Method examples are provided with the software installation.

5.1 Opening methods

To open an existing method or create a new method, choose **File:Open/New Method.**



Name	Type	Modified
Biacore Methods		
AssayDevelopment		
BufferChange	Method Builder	17-Feb-05
KineticsDiffBuffers050209	Method Builder	09-Feb-05

Select a method and click **Open** to open the method, or click **New** to create a new method. Predefined methods for common applications are provided in the folder **Biacore Methods.** If you make changes to a predefined method, you must save your changed method under a new name.

The top-level folder for methods is defined under **Tools: Preferences** (see Section 2.4). You can navigate between subfolders under the top level in the dialog box, but files outside the top-level folder are not listed in the dialog box. Click **Browse** to navigate freely in the computer file structure and open methods stored in other locations.

Check **Show importable wizard templates** to display wizard templates that can be opened in Method Builder. Opening a wizard template imports all wizard settings into a method and allows you to add functionality that is not supported in the wizard. Templates from all wizards except immobilization can be imported into Method Builder.

5.2 Method structure

Methods are handled in Method Builder in a series of sections representing different aspects of the method definition.

Overview

The **Overview** screen summarizes the method definition. Use this information as an aid in checking that the method is correctly built.

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

An assay step represents a specific function in the assay, defined in terms of what the step is intended to achieve. Assay steps may for example be start-up operations, solvent correction, sample analysis or control sample analysis. Steps can be run singly or repeated within the context of other steps: for example, start-up operations are typically performed once at the start of a run, while control samples may be repeated at intervals during the sample analysis. Analysis temperature and buffer can be set individually for each assay step.

Cycle types

Cycle types define the details of how assay steps will be performed, in terms of sample and reagent injections. Each assay step is linked to one cycle type, but the same cycle type can be used in multiple assay steps. For example, sample and control sample analysis are two assay steps that will typically use the same cycle type, ensuring that controls are analysed in exactly the same way as samples.

Parameters for injections 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 that will be run.

Report points can also be defined for each cycle type.

Sample and assay setup

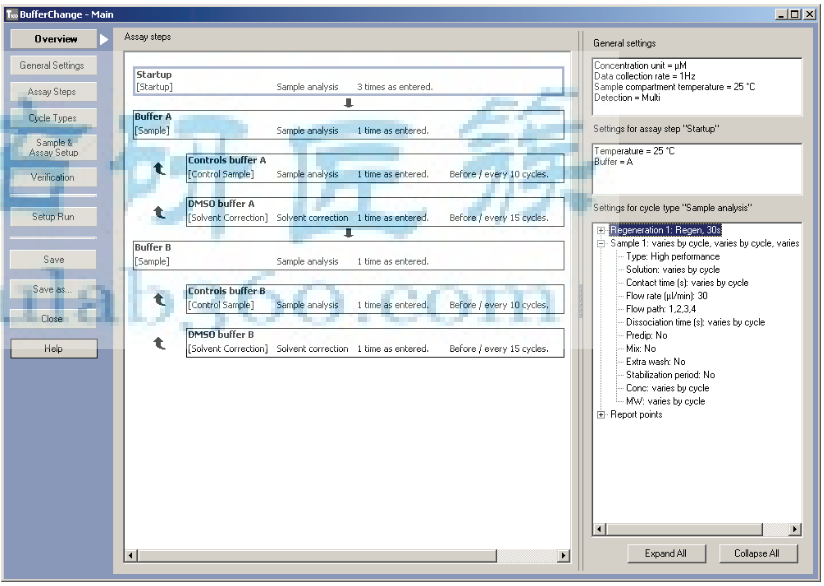
This section determines how values for variable parameters are specified. You can choose whether values are specified in the method or at run-time: this can be used to restrict the number of parameters that have to be entered when the method is run while at the same time maintaining flexibility for method development purposes.

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.

Each aspect of Method Builder is described in detail in the following sections.

5.3 Method overview



Assay steps

Startup [Startup]	Sample analysis	3 times as entered.	
Buffer A [Sample]	Sample analysis	1 time as entered.	
Controls buffer A [Control Sample]	Sample analysis	1 time as entered.	Before / every 10 cycles.
DMSO buffer A [Solvent Correction]	Solvent correction	1 time as entered.	Before / every 15 cycles.
Buffer B [Sample]	Sample analysis	1 time as entered.	
Controls buffer B [Control Sample]	Sample analysis	1 time as entered.	Before / every 10 cycles.
DMSO buffer B [Solvent Correction]	Solvent correction	1 time as entered.	Before / every 15 cycles.

General settings

Concentration unit = µM
Data collection rate = 1Hz
Sample compartment temperature = 25 °C
Detection = Multi

Settings for assay step "Startup"

Temperature = 25 °C
Buffer = A

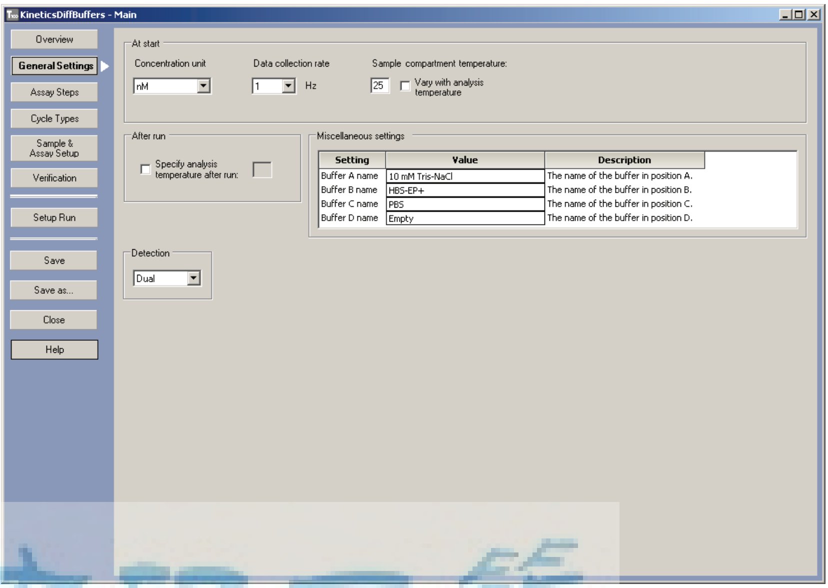
Settings for cycle type "Sample analysis"

- Regeneration 1: Regen, 30s
- Sample 1: varies by cycle, varies by cycle, varies
  - Type: High performance
  - Solution: varies by cycle
  - Contact time (s): varies by cycle
  - Flow rate (µl/min): 30
  - Flow path: 1,2,3,4
  - Dissociation time (s): varies by cycle
  - Predip: No
  - Mix: No
  - Extra wash: No
  - Stabilization period: No
  - Conc: varies by cycle
  - MW: varies by cycle
- Report points

This screen provides a summary of the method. The left panel shows the assay steps in the method (see Section 5.5). Click on an assay step to show the settings for the step and the details of the cycle definition (see Section 5.6) in the panels to the right. The cycle definition is listed as a series of injection commands: to see command details, expand individual commands by clicking on the **+**-marking or use the **Expand All** button to expand all commands in the panel.

This screen is for information only: settings cannot be changed here.

5.4 General settings



At start

Concentration unit: nM

Data collection rate: 1 Hz

Sample compartment temperature: 25 ☐ Vary with analysis temperature

After run

☐ Specify analysis temperature after run:

Miscellaneous settings

Setting	Value	Description
Buffer A name	10 mM Tris-NaCl	The name of the buffer in position A.
Buffer B name	HBS-EP+	The name of the buffer in position B.
Buffer C name	PBS	The name of the buffer in position C.
Buffer D name	Empty	The name of the buffer in position D.

Detection: Dual

The settings that are specified here are:

At start: 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.

At start: Data collection rate
Choose between 1 and 10 Hz for data collection. The higher setting will provide better resolution for kinetic analysis of fast interaction processes, but will result in larger result files.

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

After run: Analysis temperature
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 the **Vary with analysis temperature** box 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.

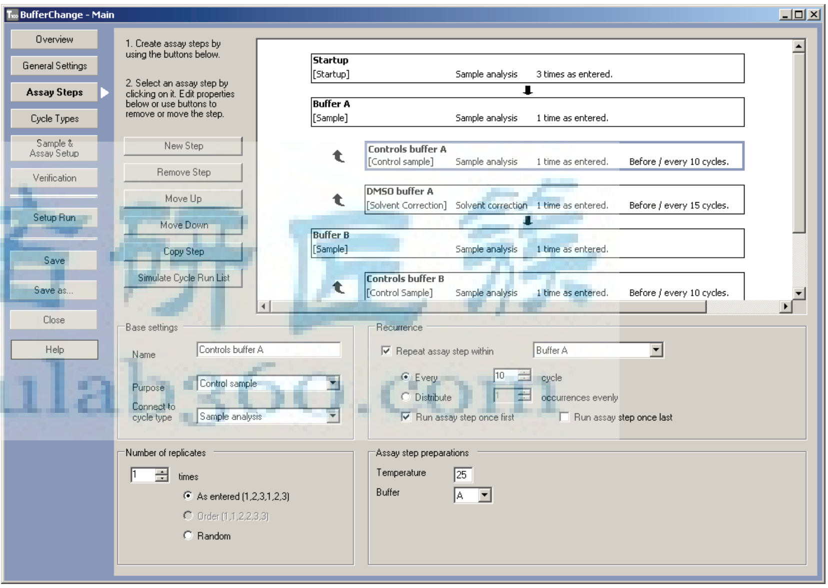
Detection
Choose between **Single** (detection in one flow cell according to the chosen flow path), **Dual** (detection in a flow cell pair according to the

chosen flow path) and **Multi** (detection in all four flow cells). This setting affects the choice of flow path that can be made for each injection command in the cycle types definitions (see Section 5.6.1).

Miscellaneous settings

Enter names if desired for the buffers in bottles A to D. Names entered here will be displayed in the **Prepare Run Protocol** (Section 4.2.6). Different buffers may be chosen for different assay steps as described in Section 5.5.4 (the default choice is bottle A).

5.5 Assay steps



1. Create assay steps by using the buttons below.

2. Select an assay step by clicking on it. Edit properties below or use buttons to remove or move the step.

Startup
[Startup] Sample analysis 3 times as entered.

Buffer A
[Sample] Sample analysis 1 time as entered.

⮤ **Controls buffer A**
[Control sample] Sample analysis 1 time as entered. Before / every 10 cycles.

⮤ **DMSO buffer A**
[Solvent Correction] Solvent correction 1 time as entered. Before / every 15 cycles.

Buffer B
[Sample] Sample analysis 1 time as entered.

⮤ **Controls buffer B**
[Control Sample] Sample analysis 1 time as entered. Before / every 10 cycles.

Base settings
Name: Controls buffer A
Purpose: Control sample
Connect to cycle type: Sample analysis

Recurrence
☑ Repeat assay step within: Buffer A
● Every 10 cycle
○ Distribute 1 occurrences evenly
☑ Run assay step once first ☐ Run assay step once last

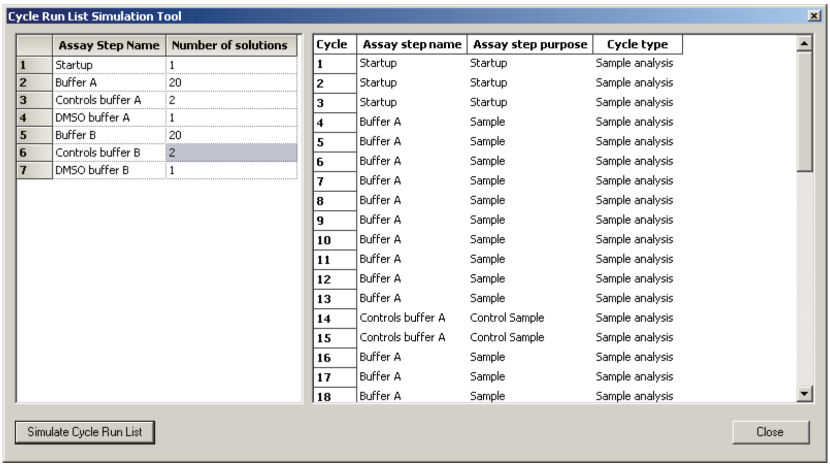
Number of replicates
1 times
● As entered (1,2,3,1,2,3)
○ Order (1,1,2,2,3,3)
○ Random

Assay step preparations
Temperature: 25
Buffer: A

This screen determines the main structure of the method in terms of assay steps. Steps at the top level (i.e. not indented or marked with the symbol ⮤) are executed in the order given. Nested steps (marked with the symbol ⮤) are executed within the context of the level in which they are placed, as specified by the settings for **Recurrence**.

To create a new assay step, click **New Step**. The step will be created with default settings at the end of the current method. Move the step in the method with the **Move Up/Down** buttons until it is in the required position in the method. You can make a copy of the currently selected step with the **Copy Step** button.

The **Simulate Cycle Run List** button beside the method summary panel allows you to check the number and order of cycles for the method as defined.



Cycle Run List Simulation Tool

	Assay Step Name	Number of solutions
1	Startup	1
2	Buffer A	20
3	Controls buffer A	2
4	DMSO buffer A	1
5	Buffer B	20
6	Controls buffer B	2
7	DMSO buffer B	1

Cycle	Assay step name	Assay step purpose	Cycle type
1	Startup	Startup	Sample analysis
2	Startup	Startup	Sample analysis
3	Startup	Startup	Sample analysis
4	Buffer A	Sample	Sample analysis
5	Buffer A	Sample	Sample analysis
6	Buffer A	Sample	Sample analysis
7	Buffer A	Sample	Sample analysis
8	Buffer A	Sample	Sample analysis
9	Buffer A	Sample	Sample analysis
10	Buffer A	Sample	Sample analysis
11	Buffer A	Sample	Sample analysis
12	Buffer A	Sample	Sample analysis
13	Buffer A	Sample	Sample analysis
14	Controls buffer A	Control Sample	Sample analysis
15	Controls buffer A	Control Sample	Sample analysis
16	Buffer A	Sample	Sample analysis
17	Buffer A	Sample	Sample analysis
18	Buffer A	Sample	Sample analysis

Enter the number of cycles to be run in each assay step and then click **Simulate Cycle Run List** to display the cycle sequence for the run as currently defined.

5.5.1 Base settings

Name

This is the name of the assay step. Each step in a method must have a unique name. New steps are by default named **Assay Step n**, where **n** is a serial number: change the name to something that describes the context or intent of the step, to make the method easier to follow.

Purpose

Assay steps are assigned a purpose, used to identify cycles in the evaluation software. The choice of purpose can help to document the method structure, and also determines the way the data is treated in evaluation. Choose the purpose from the list.

An assay step may have one of the following purposes:

Purpose	Usage and restrictions
Calibration	Used for calibration curves in concentration assays and affinity in solution. 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. Set **Calibration** to recurring within **Sample** to repeat the calibration at intervals through the assay. At least one calibration step is required for evaluation of concentration analysis.
Conditioning	Used to condition the sensor surface at the start of an assay.

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. Set **Control sample** to recurring within **Sample** to repeat the control sample analysis at intervals through the assay.
Sample	Used for sample analysis in all applications. At least one sample step is required for application-specific evaluation.
Solvent correction	Used for solvent correction cycles. This step should be connected to a cycle type designed for solvent correction. Set **Solvent correction** to recurring within **Sample** to repeat the solvent correction at intervals through 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 simple 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 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.

Connect to cycle type
Each assay step is connected to one cycle type, which determines the detailed operation of the step (see Section 5.6). Choose the cycle type from the list of types available in the method.

5.5.2 Number of replicates

Assay steps can be set to run in replicate, which means that all cycles in the assay step will be repeated the specified number of times. The order in which cycles in the assay step are repeated can be specified:

As entered	performs all cycles in the step once, then repeats the step until the number of replicates is reached (this is represented as **1,2,3,1,2,3** to illustrate the order of 3 cycles in a step repeated twice)

Order	performs the first cycle in the step for the specified number of replicates, then the second cycle and so on (represented as **1,1,2,2,3,3**)
Random	randomizes the order of the cycles within the step until all cycles have been executed the specified number of times.

Any steps that recur in a replicated step are performed at the specified recurrence interval (see below) within the total number of cycles in the replicates of the step. The number of replicates for the recurring step is set in the properties of that step, and is not affected by the number of replicates at the higher level.

5.5.3 Recurrence

An assay step can be set to recur at a specified interval within the context of another step. The recurrence can be specified as **Every *n* cycles** (so that the number of occurrences will depend on the number of cycles in the assay step) or **Distribute *n* occurrences evenly** (in which case the number of occurrences is fixed and they are distributed as evenly as possible among the cycles in the assay step. In addition, the recurring step can be specified to be executed at the beginning and/or end of the step in which it is set to recur.

If the higher level step is run in replicate, a recurring step is distributed among the total number of cycles including replicates. This is illustrated by the calculations below for a recurring step set to **Every 5 cycles**:

Top level step			
Number of cycles	10	20	10
Number of replicates	1	1	2
Total number of cycles	10	20	20
Number of recurrences for the nested step	2	4	4

5.5.4 Assay step preparations

Temperature

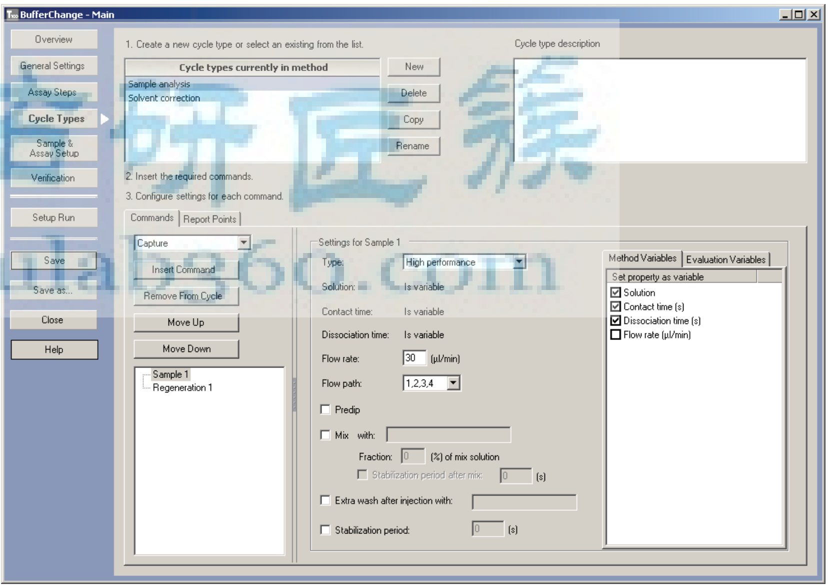
This value determines the analysis temperature for the assay step. The setting will also control the sample compartment temperature if the appropriate option is checked under **General Settings** (Section 5.4).

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 setting is reached.

Buffer

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).

5.6 Cycle types



1. Create a new cycle type or select an existing from the list.

Cycle types currently in method
Sample analysis
Solvent correction

2. Insert the required commands.

3. Configure settings for each command.

Capture

Sample 1
Regeneration 1

Settings for Sample 1
Type: High performance
Solution: Is variable
Contact time: Is variable
Dissociation time: Is variable
Flow rate: 30 (µl/min)
Flow path: 1,2,3,4
☐ Predip
☐ Mix with:
Fraction: 0 (%) of mix solution
☐ Stabilization period after mix: 0 (s)
☐ Extra wash after injection with:
☐ Stabilization period: 0 (s)

Set property as variable
☑ Solution
☑ Contact time (s)
☑ Dissociation time (s)
☐ Flow rate (µl/min)

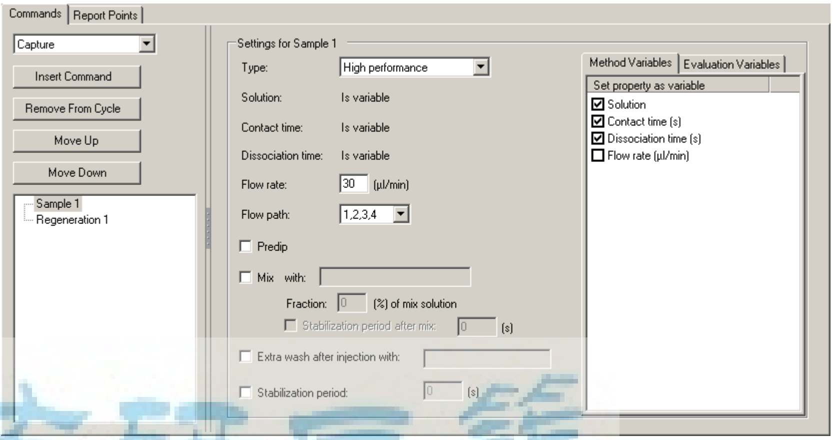
Cycle types define the detailed sequence of operations to be performed in each assay step.

The top panel in the work area lists the cycle types currently available in the method. Use the **New** button to create a new cycle type. Mark a cycle type and click **Delete** to remove the cycle type from the method. Use the **Copy** button to make copies of cycle types in the method: this can be useful if a method requires a number of similar cycle types with small variations. Click **Rename** to rename a cycle type.

Enter a description of the cycle type if desired.

Settings for the currently selected cycle type are configured in the lower part of the work area. The settings are divided into **Commands** and **Report Points**, accessed on the respective tabs.

5.6.1 Commands



Settings for Sample 1

Type: High performance

Solution: Is variable

Contact time: Is variable

Dissociation time: Is variable

Flow rate: 30 (µl/min)

Flow path: 1,2,3,4

☐ Predip

☐ Mix with:

Fraction: 0 (%) of mix solution

☐ Stabilization period after mix: 0 (s)

☐ Extra wash after injection with:

☐ Stabilization period: 0 (s)

☑ Solution
☑ Contact time (s)
☑ Dissociation time (s)
☐ Flow rate (µl/min)

The commands in a cycle definition correspond to different kinds of injection of liquid over the sensor surface. To add a new command to the cycle definition, choose the command type from the pull-down list and click **Insert command.** The command will be inserted with default parameter settings immediately after the currently marked command (or at the end of the cycle definition if no command is marked). Use the **Move up** and **Move down** buttons to change the position of the command in the cycle. Commands are executed from top to bottom in the cycle definition.

General command properties

Common features of several commands are check-boxes for **Predip, Extra Wash After Injection** and **Stabilization period.**

Predip

Check this box to dip the needle in a separate position before aspirating the solution to be injected. The predip position will normally contain the same solution as is injected, so that the needle is rinsed briefly to minimize carry-over effects. The same predip position is used for all cycles.

Extra Wash After Injection

Check this box 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 box and specify a time in seconds to introduce a delay before the next command is started. This can sometimes be necessary (for example after regeneration steps) to allow the response to stabilize before performing the next injection.

Capture command

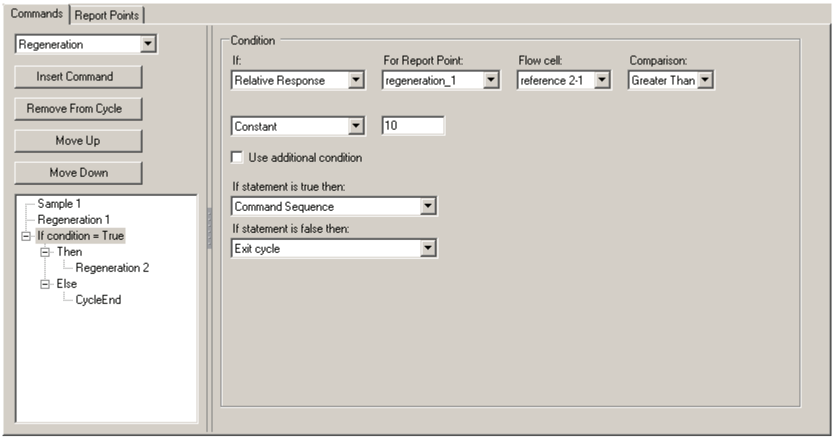
This command is intended for injection of ligand over a capturing molecule at the beginning of a cycle. The injected solution, contact time and flow rate can be set as variables.

Carry-over control command

This command 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 the **If...then** command) to perform additional buffer injections or regeneration steps if carry-over is detected. A plot of the binding response from a carry-over injection against cycle number is created automatically for quality control purposes in the evaluation software (see Section 6.4).

If...then command

This command allows construction of conditional methods, where commands 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:



Regeneration

- Sample 1
- Regeneration 1
- If condition = True
  - Then
    - Regeneration 2
  - Else
    - CycleEnd

If: Relative Response
For Report Point: regeneration_1
Flow cell: reference 2-1
Comparison: Greater Than
Constant 10
☐ Use additional condition
If statement is true then: Command Sequence
If statement is false then: Exit cycle

To set up a conditional command:

1. Insert an **If...then** command 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 either AND (both conditions must be fulfilled) or OR (fulfillment of one condition is sufficient) as a logical operator.

   (The available comparison conditions are **Greater than** and **Less than.** The conditions do not include **Equal to** since exact equality is an unpredictable condition in view of noise in the SPR response. To construct an equality condition, combine one **Greater than** and one **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 a command sequence, terminate the cycle or the method, or introduce a stabilization period.

   If you choose a command sequence for either the **True** or **False** outcome, click on the appropriate branch of the command (**Then** or **Else** respectively) and insert the commands you wish to be executed. If you leave the branch empty, the cycle will simply continue with the next command following the **If...then** construction.

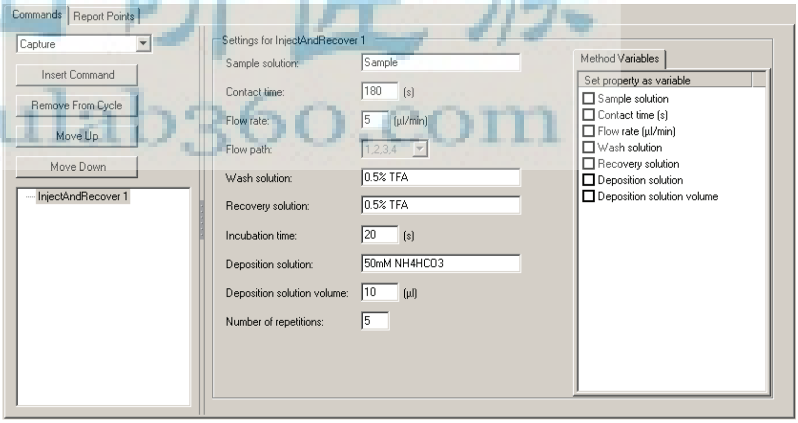
   You can use the **Move up** and **Move down** buttons to rearrange the order of commands within a branch, but you cannot move commands outside the branch in which they are placed.

   If you have chosen a command sequence for an action and have entered commands, you must delete the commands before you can change to a different action.

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

General command
This command is a general-purpose injection that supports the same functions as the **Sample** command, and also includes a **Dual Inject** option under **Type**. This option injects two solutions in direct succession, with no intervening automatic wash routines. A dissociation time may be set for the second injection but not for the first. **General** commands are not recognized as analyte injections for evaluation of concentration, kinetics or affinity and may therefore also be used to “hide” injections from the predefined evaluation facilities. The injected solution, contact time and flow rate can be set as variables. Evaluation variables can also be defined for **General** commands (see Section 5.6.2).

Inject and recover command
This command recovers analyte that is bound to the sensor surface, and is intended for use in applications where the bound material is analyzed further. Several features of the command are designed specifically for integration of Biacore analysis with mass spectrometry (MS). Most of the parameters for the command can be set as variables. This command can only be used for injection over all four flow cells: normally, the same ligand should be immobilized in each cell.



Settings for InjectAndRecover 1

Sample solution: Sample
Contact time: 180 (s)
Flow rate: 5 (µl/min)
Flow path: 1,2,3,4
Wash solution: 0.5% TFA
Recovery solution: 0.5% TFA
Incubation time: 20 (s)
Deposition solution: 50mM NH4HCO3
Deposition solution volume: 10 (µl)
Number of repetitions: 5

Set property as variable
☐ Sample solution
☐ Contact time (s)
☐ Flow rate (µl/min)
☐ Wash solution
☐ Recovery solution
☐ Deposition solution
☐ Deposition solution volume

The command initiates a sequence of operations in the instrument:

1. The specified volume of **Deposition solution** is transferred in the autosampler to a target position in the sample and reagent rack. Target positions are assigned as required in the **Rack Positions** dialog (Section 4.2.5). The deposition solution should be MS-compatible, and may be used for example to neutralize the recovery conditions (which are often acidic) or to add trypsin or another protease to the sample for peptide digestion. The presence of deposition solution also helps to collect the small recovered volume reliably from the autosampler needle.

2. The **Sample solution** is injected over the sensor surface with the **Contact time** and **Flow rate** as specified. The **Flow path** is fixed so that sample passes through all four flow cells to maximize the amount of analyte that binds to the surface.

3. The flow system is washed with the specified **Wash solution**. Distilled water or an MS-compatible buffer should be used as washing solution.

4. A small volume (approximately 2 µl) of **Recovery solution**, separated from the surrounding buffer by air segments, is injected into the flow cells. The flow is stopped for the specified **Incubation time** while the recovery solution is in contact with the sensor surface, to allow the bound analyte to dissociate into the recovery solution.

5. The flow direction over the sensor surface is reversed and the recovery solution containing recovered analyte is deposited in the target position where it mixes passively with the deposition solution.

6. Steps 2-5 are repeated for the specified **Number of repetitions**. This increases the yield of recovered analyte without requiring additional commands. The same target position is used for recovered analyte from all repetitions. Note that only one aliquot of deposition solution is used, regardless of the number of repetitions.

Notes: Methods that include the **InjectAndRecover** command require a sample and reagent rack type 1 and cannot be used with microplates (see the Biacore T100 Instrument Handbook for rack details).

The contact time for sample, flow rate and number of repetitions determine the total injected volume for both sample and recovery solution. You may need to adjust one or more of these parameters if the method does not pass verification.

Regeneration command

This command 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 for higher viscosity. The injected solution, contact time and flow rate can be set as variables.

Sample command
This command is intended for injection of sample containing analyte. Only **Sample** commands are recognized in the evaluation software for kinetics, affinity and concentration evaluation. The injected solution, contact time, dissociation time and flow rate can be set as variables. Evaluation variables can also be defined for the **Sample** command (see Section 5.6.2).

The **Sample** command may be optimized for high performance or low sample consumption. High performance is achieved by using extra segments of air and sample during aspiration to separate the injected solution from running buffer, thereby minimizing dispersion of sample at the beginning and end of the injection at the expense of additional 25 µl sample consumption. The low sample consumption option uses fewer segments, but still achieves a performance that is adequate for all applications except analysis of rapid kinetics.

The **Sample** command supports 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 autosampler 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.

Solvent correction command
This command injects a 30-second pulse of solvent correction solution over the surface at a flow rate of 30 µl/min. A solvent correction cycle should contain 4-8 **Solvent correction** commands for the different solvent concentrations used to construct the correction curve (see Section 6.7). **Solvent correction** commands will be correctly evaluated only when they are used in cycle types that are connected to assay steps with purpose **Solvent correction.**

5.6.2 Variables

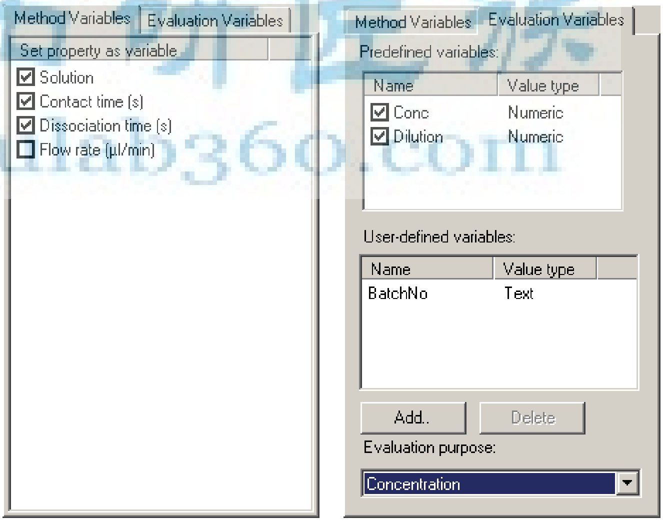
Parameters for several of the commands in a cycle definition may be set as variables. Values for variables are entered in either the **Sample and Assay Setup** or the **Setup Run** step (Sections 5.7 and 5.9.2), and determine the number of cycles that will be performed in the run. Variables fall into two broad classes:

- *Method variables* such as sample name or contact time, that control the way the cycle will be performed. Parameters that are not set as variables are defined in the main command panel.

- ***Evaluation variables*** such as concentration or analyte molecular weight that are used in evaluation of the data. 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. 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 (an example might be the sample batch number). Evaluation variables may only be defined for **Sample** and **General** commands.

Variables are set in the list at the right of the command panel. For method variables, check a parameter to set it as variable. For evaluation variables, choose the purpose of the assay to display an appropriate list of predefined variables and check the variables you want to use. Click **Add** to set up user-defined variables.

Note: For specific assay purposes, you should generally check all suggested variables. If you leave some variables unchecked, the assay-specific evaluation may not work.



Set property as variable

☑ Solution
☑ Contact time (s)
☑ Dissociation time (s)
☐ Flow rate (µl/min)

Predefined variables:

Name	Value type
☑ Conc	Numeric
☑ Dilution	Numeric

User-defined variables:

Name	Value type
BatchNo	Text

Evaluation purpose: Concentration

Predefined evaluation variables for different assay purposes are described in the table below (see also Section 5.10).

Assay purpose: General, Kinetics/Affinity, Thermodynamics

Conc	Analyte concentration
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.

Assay purpose: 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.

Assay purpose: Concentration

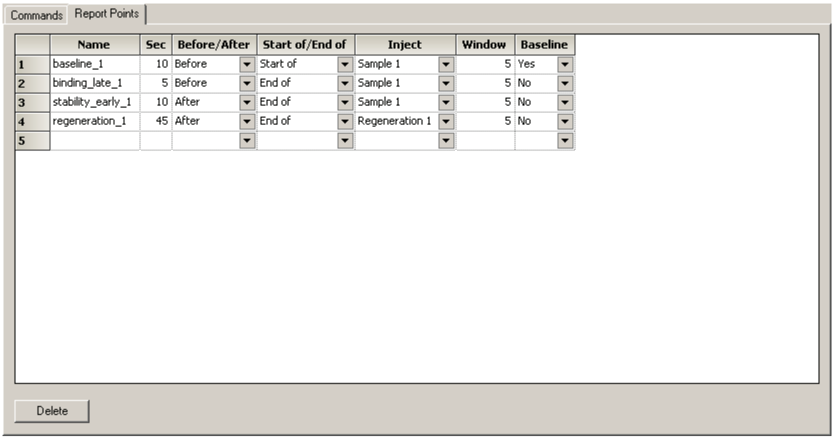
Conc	Analyte concentration: required for calibration and control samples, left blank for unknowns
Dilution	Dilution factor: used for unknown samples to calculate original concentrations

*Assay purpose: Affinity in solution**

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)

*See Section 11.1.1 for details of how this assay is set up.

5.6.3 Report points



	Name	Sec	Before/After	Start of/End of	Inject	Window	Baseline
1	baseline_1	10	Before	Start of	Sample 1	5	Yes
2	binding_late_1	5	Before	End of	Sample 1	5	No
3	stability_early_1	10	After	End of	Sample 1	5	No
4	regeneration_1	45	After	End of	Regeneration 1	5	No
5							

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 injection commands have a predefined set of report points that are added to the list when the command 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.

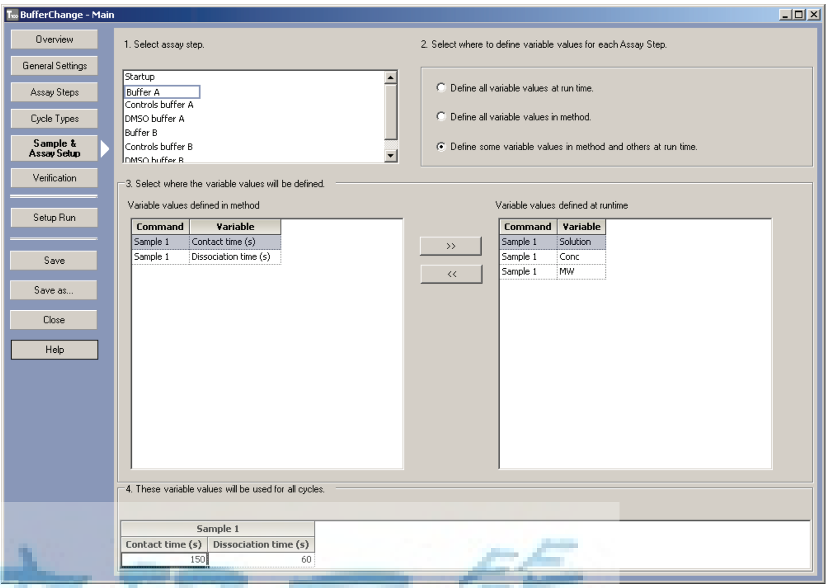
Report points are set at defined times in relation to the start or end of injections in the cycle. Report points that are set outside the time range for the cycle (i.e. a significant time before the start of the first injection or after the end of the last injection) will not be created.

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 details as follows:

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.
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 seconds 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.

5.7 Sample and Assay Setup



1. Select assay step.

Startup
Buffer A
Controls buffer A
DMSO buffer A
Buffer B
Controls buffer B
DMSO buffer B

2. Select where to define variable values for each Assay Step.

○ Define all variable values at run time.
○ Define all variable values in method.
● Define some variable values in method and others at run time.

3. Select where the variable values will be defined.

Variable values defined in method

Command	Variable
Sample 1	Contact time (s)
Sample 1	Dissociation time (s)

Variable values defined at runtime

Command	Variable
Sample 1	Solution
Sample 1	Conc
Sample 1	MW

4. These variable values will be used for all cycles.

Sample 1	
Contact time (s)	Dissociation time (s)
150	60

This screen determines whether variable parameter values are specified in the method or at run time. You may choose to specify all values in the method, all at run time, or a mixture of the two. Values that are specified in the method are saved with the method and cannot be changed at run time. You can change the level at which values are specified without changing the cycle type definition, so that the same cycle type can be used for different assay steps with different sets of run-time variables. An example of this may be found in the predefined method for affinity in solution (Section 5.11.1).

Variables are configured independently for the different assay steps (even if the assay steps use the same cycle type).

If you choose to specify all variables in the method, the values are entered in this screen. For each assay step, one row of variable values represents one analysis cycle (the cycle may be repeated if the **Repeat** property is set in the **Assay Step** screen). 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 commands in the cycle type definition. Use the right mouse button in the variables table to access functions for copying and pasting cell contents and for inserting and removing rows. When all variables are specified at run time, variables are handled in the same way in the **Setup Run** step.

To specify that some variables are specified in the method and others at run time, check the appropriate option and then distribute the variables as required between the method and run-time lists. This mode can be used to hide variables at run time that are not relevant for the assay step. Fill in values for variables that are specified in the method: those specified at run time are filled in the **Setup Run** step. Note that in this mode only one set of variable values can be specified in the method: the number of cycles is determined by the number of rows of variable values in **Setup Run**.

Depending on how the method is defined, there may be variable tables for several assay steps. Variable handling must be defined for all steps before the method will pass verification.

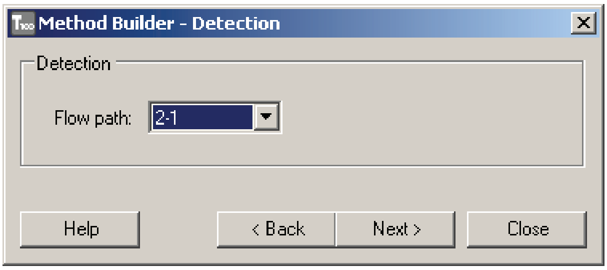
5.8 Verification

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).

5.9 Setup Run

5.9.1 Detection

Set the flow path for the method in the **Detection** dialog.



Method Builder - Detection

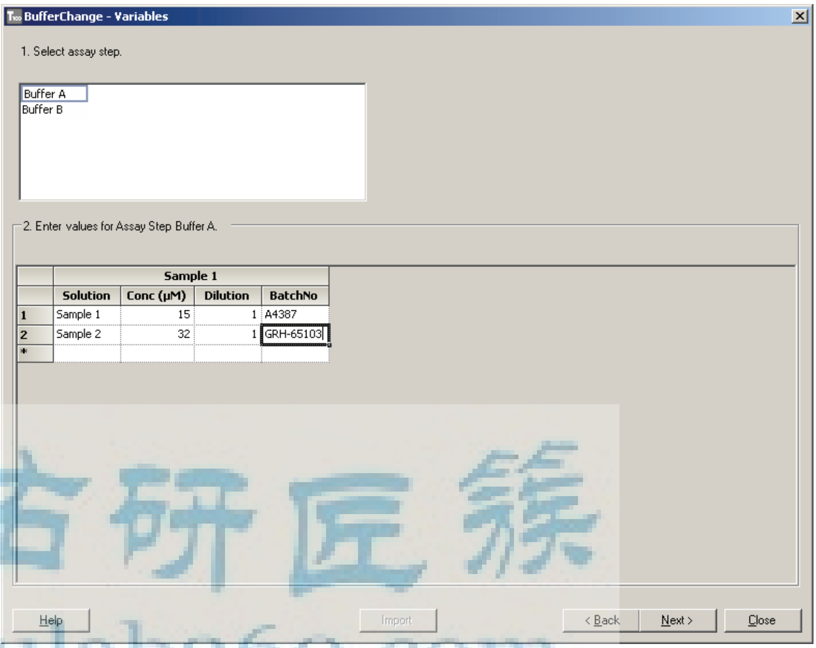
Detection

Flow path: 2-1

You can only choose a flow path that is consistent with the **Detection** setting for the method (see Section 5.4).

5.9.2 Variables

In this step you assign values to variables that are to be defined at run time (typically a sample table, see Section 5.7). 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 commands in the cycle type definition.



1. Select assay step.

Buffer A
Buffer B

2. Enter values for Assay Step Buffer A.

	Sample 1			
	Solution	Conc (µM)	Dilution	BatchNo
1	Sample 1	15	1	A4387
2	Sample 2	32	1	GRH-65103
*				

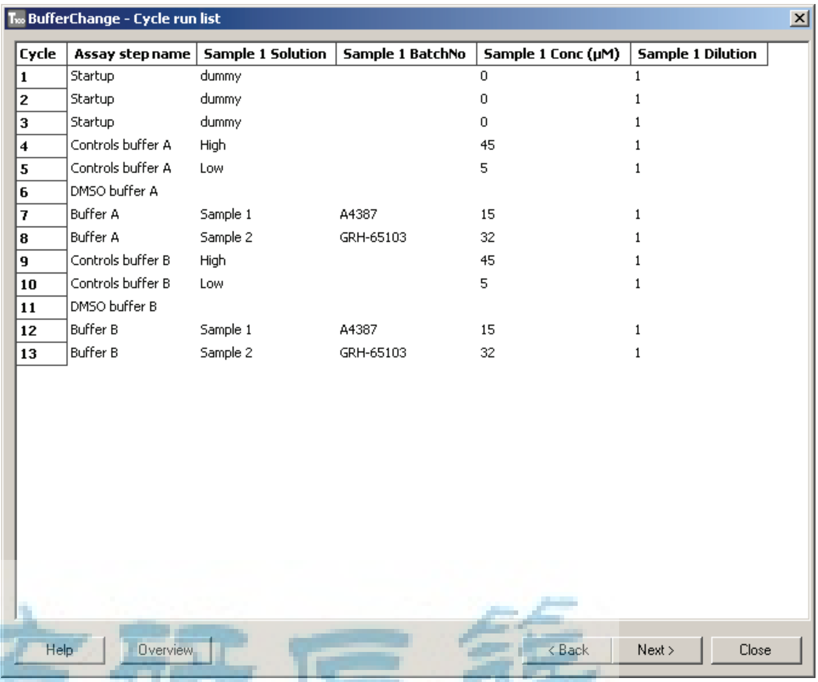
Use the right mouse button in the variables table to access functions for copying and pasting cell contents and for inserting and removing rows. The columns in the table are listed in the order they are defined in the method (see Section 5.6.2).

Click **Import** to import the variables table from an external file. See Appendix A for details of the import format.

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

5.9.3 Cycle run list

When you have completed the variables table, 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 variable tables are correctly filled in.

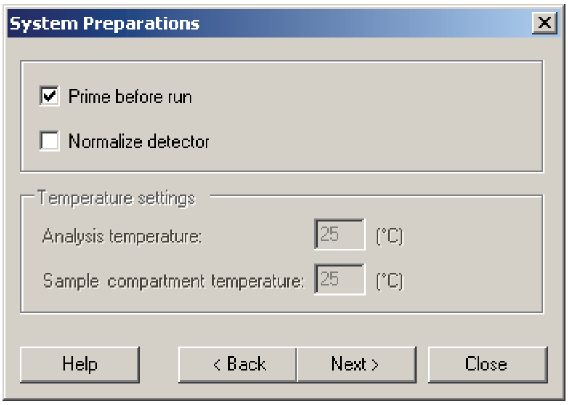


Cycle	Assay step name	Sample 1 Solution	Sample 1 BatchNo	Sample 1 Conc (µM)	Sample 1 Dilution
1	Startup	dummy		0	1
2	Startup	dummy		0	1
3	Startup	dummy		0	1
4	Controls buffer A	High		45	1
5	Controls buffer A	Low		5	1
6	DMSO buffer A				
7	Buffer A	Sample 1	A4387	15	1
8	Buffer A	Sample 2	GRH-65103	32	1
9	Controls buffer B	High		45	1
10	Controls buffer B	Low		5	1
11	DMSO buffer B				
12	Buffer B	Sample 1	A4387	15	1
13	Buffer B	Sample 2	GRH-65103	32	1

Click **Overview** to display the method overview (Section 5.3).

5.9.4 System preparations

Choose which preparation steps should be executed before the method starts.



☑ Prime before run

☐ Normalize detector

Temperature settings

Analysis temperature: 25 (°C)

Sample compartment temperature: 25 (°C)

System preparations are equivalent to those for wizard-based runs (see Section 4.2.4). The temperature settings are taken from the first assay step in the method and cannot be changed here: they are shown for information only.

5.9.5 Rack positions

See Section 4.2.5 for a description of the **Rack positions** dialog box.

5.9.6 Prepare Run Protocol

See Section 4.2.6 for a description of the **Prepare Run Protocol** dialog box.

5.9.7 Starting the run

When the positions are finalized, the sample plate is prepared and loaded into the instrument and a chip is docked, choose **Start Run** to start the run.

5.10 Requirements for assay-specific evaluation

This section describes the requirements and recommendations if assay-specific evaluation is to be applied to method-based runs.

5.10.1 Concentration

See Chapter 8 for a description of concentration evaluation.

- At least one assay step is required with purpose **Calibration** and one with purpose **Sample**. An assay step with purpose **Control Sample** is also required in order to create trend plots for control samples.

- Assay steps **Calibration**, **Sample** and **Control Sample** must be connected to a cycle type that includes one **Sample** command. The three assay steps will normally be connected to the same cycle type.

- Samples in the **Calibration** step must have concentrations specified in the variable **Conc**. At least two different concentrations are required for linear calibration curves and at least four for 4-parameter fitting.

- Samples in the **Sample** step will normally not have specified concentrations. If concentrations are specified, they will be ignored.

5.10.2 Kinetics/Affinity

See Chapter 9 for a description of kinetics and affinity evaluation.

- At least one assay step is required with purpose **Sample**, connected to a cycle type that includes one **Sample** command.

- 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**.

- As a recommendation, there should be a concentration series with at least five non-zero analyte concentrations and one zero concentration. At least one of the non-zero concentrations should be measured in duplicate. Although kinetic and affinity evaluation can be applied to runs with fewer sensorgrams, the results will generally be less reliable if these recommendations are not followed.

5.10.3 Thermodynamics

See Chapter 10 for a description of thermodynamics evaluation.

Thermodynamics evaluation requires that kinetics or affinity (see Section 5.10.2) is determined at two or more temperatures.

5.10.4 Affinity in solution

See Chapter 11 for a description of affinity in solution evaluation.

- 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** command. 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.

5.10.5 Other requirements

Application of solvent correction (see Section 6.7) requires an assay step with purpose **Solvent Correction**, connected to a cycle type that includes at least four **Solvent Correction** commands for different solvent concentrations.

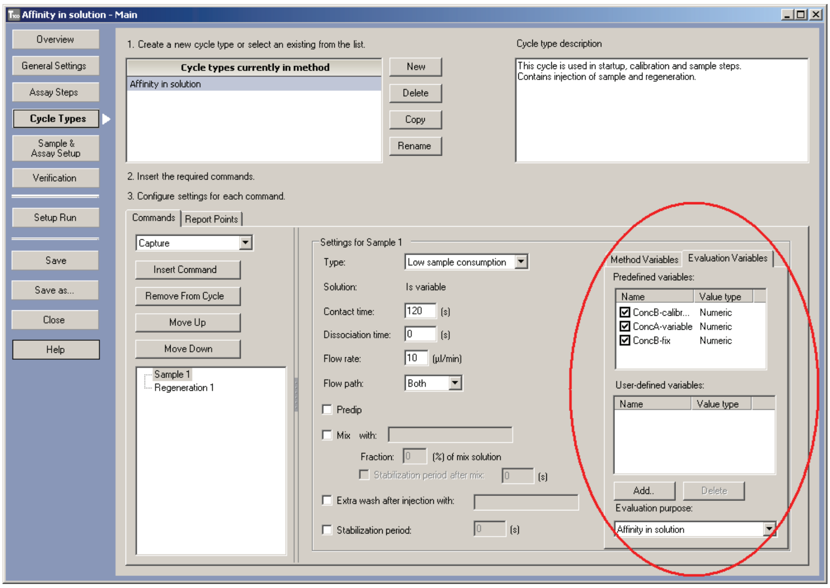
5.11 Method examples

A selection of predefined methods covering common applications is provided in the **Biacore Methods** folder (see Section 5.1). Use these methods either directly or as starting points for your own method development. This section describes the essential features in each method that are not supported in wizards. Refer to these methods as guidelines in constructing your own methods that exploit similar features.

5.11.1 Affinity in solution

This method is designed for measurement of affinity in solution as described in Chapter 11. The method includes a **Calibration** assay step for measurement of component B and a **Sample** step for measurement of mixtures of components A and B. Both these assay steps are connected to the same cycle type.

Predefined evaluation variables are included for the evaluation purpose **Affinity in solution**:



1. Create a new cycle type or select an existing from the list.

Cycle types currently in method
Affinity in solution

Cycle type description: This cycle is used in startup, calibration and sample steps. Contains injection of sample and regeneration.

2. Insert the required commands.

3. Configure settings for each command.

Capture

Sample 1
Regeneration 1

Settings for Sample 1
Type: Low sample consumption
Solution: Is variable
Contact time: 120 (s)
Dissociation time: 0 (s)
Flow rate: 10 (µl/min)
Flow path: Both
☐ Predip
☐ Mix with:
Fraction: 0 (%) of mix solution
☐ Stabilization period after mix: 0 (s)
☐ Extra wash after injection with:
☐ Stabilization period: 0 (s)

Predefined variables:

Name	Value type
☑ ConcB-calibr...	Numeric
☑ ConcA-variable	Numeric
☑ ConcB-fix	Numeric

User-defined variables:

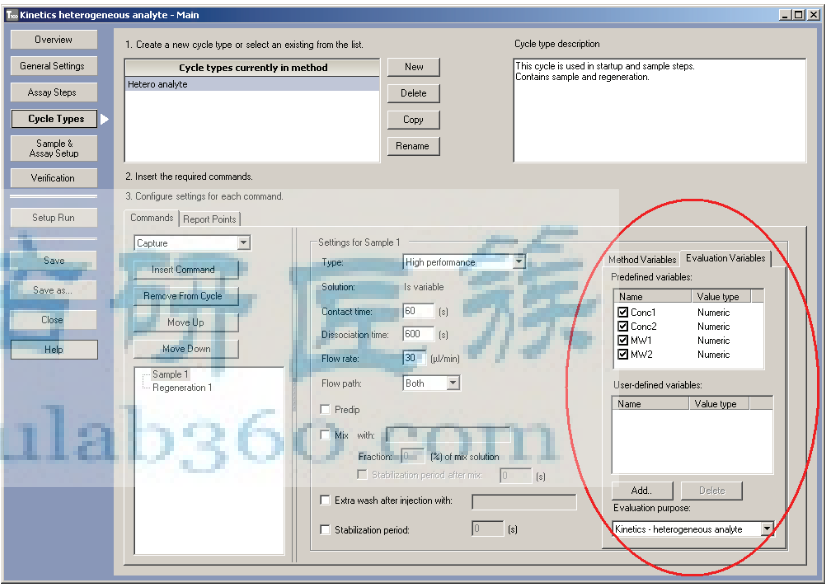
Name	Value type

Evaluation purpose: Affinity in solution

The **Sample and Assay Setup** is different for the two assay steps, so that only the relevant variables are entered at run-time for each assay step.

5.11.2 Heterogeneous analyte kinetics

This method is a straightforward kinetics determination with evaluation variables included for the evaluation purpose **Kinetics – heterogeneous analyte**, providing separate concentration and molecular weight variables for two analytes.



Kinetics heterogeneous analyte - Main

1. Create a new cycle type or select an existing from the list.

Cycle types currently in method
Hetero analyte

Cycle type description: This cycle is used in startup and sample steps. Contains sample and regeneration.

2. Insert the required commands.

3. Configure settings for each command.

Settings for Sample 1

Type: High performance
Solution: Is variable
Contact time: 60 (s)
Dissociation time: 600 (s)
Flow rate: 30 (µl/min)
Flow path: Both
☐ Predip
☐ Mix with:
Fraction: 0 (%) of mix solution
☐ Stabilization period after mix: 0 (s)
☐ Extra wash after injection with:
☐ Stabilization period: 0 (s)

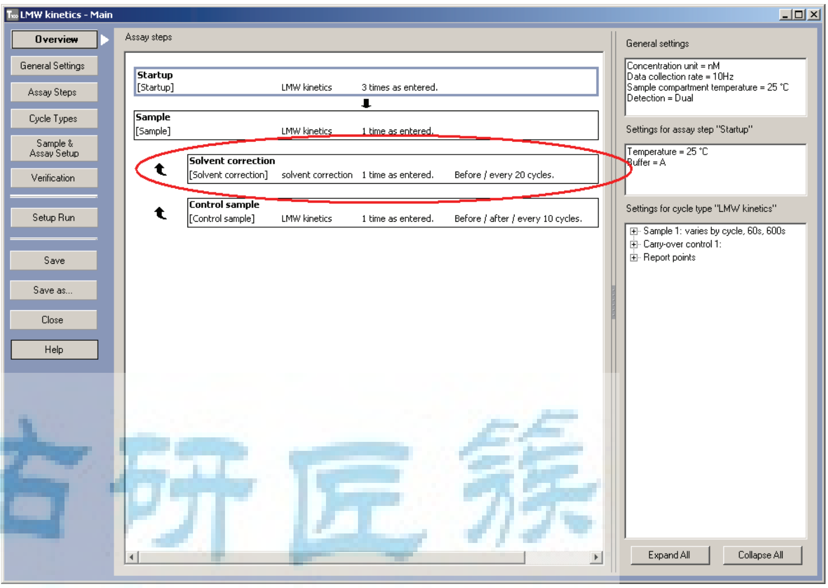
Predefined variables:

Name	Value type
☑ Conc1	Numeric
☑ Conc2	Numeric
☑ MW1	Numeric
☑ MW2	Numeric

Evaluation purpose: Kinetics - heterogeneous analyte

5.11.3 Low molecular weight compounds

Methods are provided for both kinetics and screening of low molecular weight compounds. The essential addition to wizard-based counterparts is the inclusion of a **Solvent Correction** assay step repeated at interval throughout the run.



Assay steps

Startup
[Startup] LMW kinetics 3 times as entered.

Sample
[Sample] LMW kinetics 1 time as entered.

Solvent correction
[Solvent correction] solvent correction 1 time as entered. Before / every 20 cycles.

Control sample
[Control sample] LMW kinetics 1 time as entered. Before / after / every 10 cycles.

General settings

Concentration unit = nM
Data collection rate = 10Hz
Sample compartment temperature = 25 °C
Detection = Dual

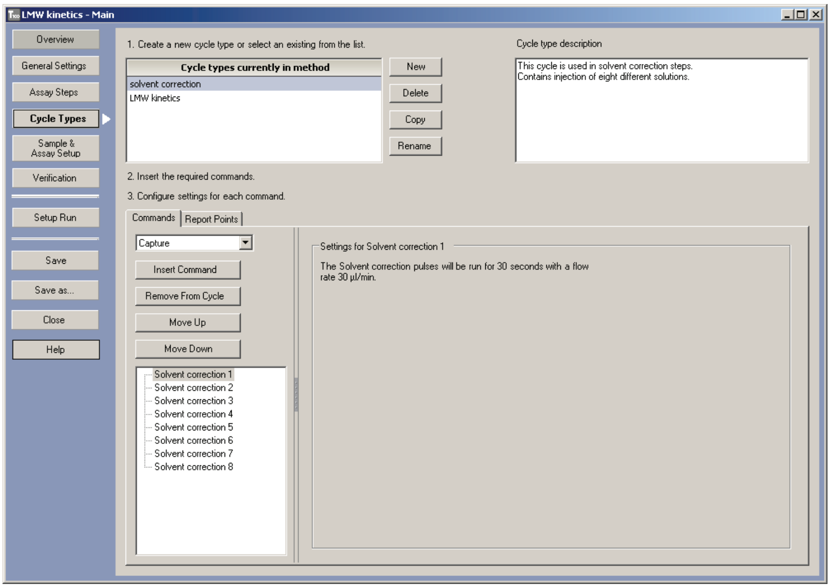
Settings for assay step "Startup"

Temperature = 25 °C
Buffer = A

Settings for cycle type "LMW kinetics"

- Sample 1: varies by cycle, 60s, 600s
- Carry-over control 1:
- Report points

This assay step is connected to a cycle type that includes 8 **Solvent Correction** commands for injection of 8 different solvent concentrations (see Section 6.7).



1. Create a new cycle type or select an existing from the list.

Cycle types currently in method
solvent correction
LMW kinetics

Cycle type description

This cycle is used in solvent correction steps.
Contains injection of eight different solutions.

2. Insert the required commands.

3. Configure settings for each command.

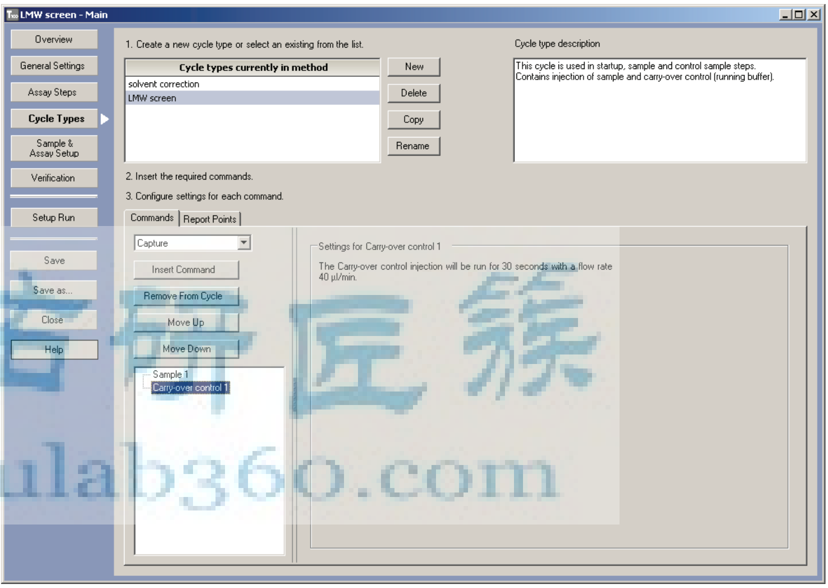
Solvent correction 1
Solvent correction 2
Solvent correction 3
Solvent correction 4
Solvent correction 5
Solvent correction 6
Solvent correction 7
Solvent correction 8

Settings for Solvent correction 1

The Solvent correction pulses will be run for 30 seconds with a flow rate 30 µl/min.

5.11.4 Screening

Screening applications suitably include a carry-over control injection, to identify potential carry-over problems from “sticky” compounds that may affect the response in subsequent injections. The Biacore method is designed for screening of low molecular weight compounds and includes a solvent correction step as described in Section 5.11.3. Low molecular weight compounds frequently dissociate readily from their targets, and the example method does not include regeneration. If you require a regeneration step, it is advisable to include regeneration after both the sample and the carry-over injections.



1. Create a new cycle type or select an existing from the list.

Cycle types currently in method
solvent correction
LMW screen

Cycle type description

This cycle is used in startup, sample and control sample steps.
Contains injection of sample and carry-over control (running buffer).

2. Insert the required commands.

3. Configure settings for each command.

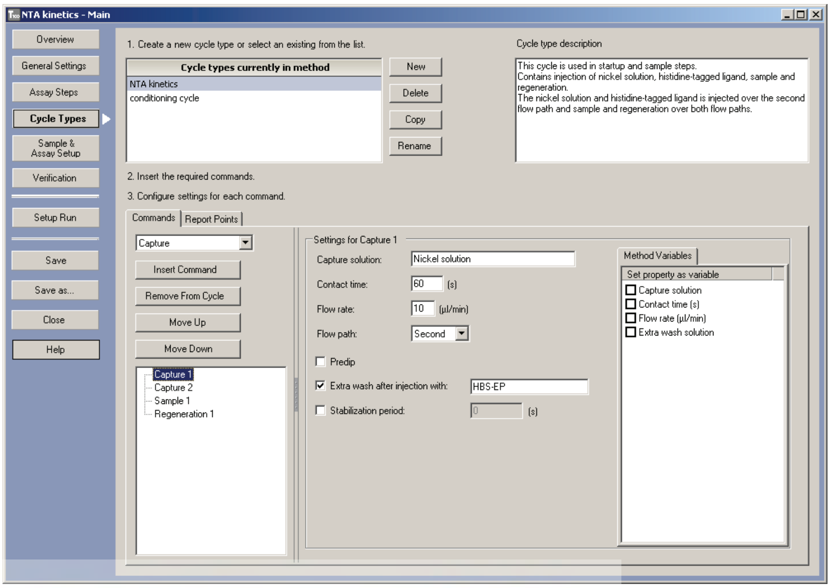
Sample 1
Carry-over control 1

Settings for Carry-over control 1

The Carry-over control injection will be run for 30 seconds with a flow rate 40 µl/min.

5.11.5 Capture on Sensor Chip NTA

Capture of poly-histidine tagged ligands on Sensor Chip NTA relies on chelation of Ni^{2+} ions on the surface, and regeneration is generally performed by stripping the Ni^{2+} with EDTA. Applications that use this approach require two capture injections in each cycle, one for Ni^{2+} and one for the poly-His tagged ligand. The Biacore method provided is designed for kinetic or affinity determinations, but may be readily modified for other applications that use Sensor Chip NTA.



1. Create a new cycle type or select an existing from the list.

Cycle types currently in method
NTA kinetics
conditioning cycle

Cycle type description
This cycle is used in startup and sample steps.
Contains injection of nickel solution, histidine-tagged ligand, sample and regeneration.
The nickel solution and histidine-tagged ligand is injected over the second flow path and sample and regeneration over both flow paths.

2. Insert the required commands.

3. Configure settings for each command.

Capture

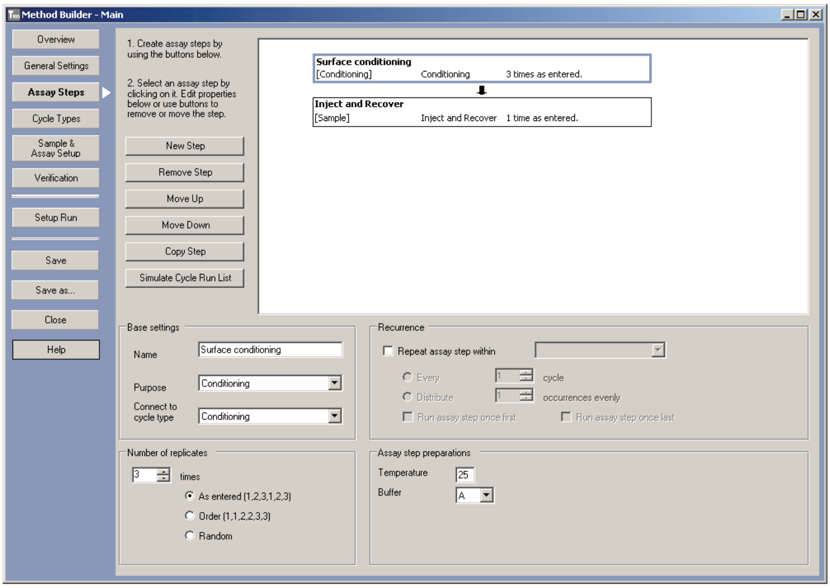
- Capture 1
- Capture 2
- Sample 1
- Regeneration 1

Settings for Capture 1
Capture solution: Nickel solution
Contact time: 60 (s)
Flow rate: 10 (µl/min)
Flow path: Second
☐ Predip
☑ Extra wash after injection with: HBS-EP
☐ Stabilization period: 0 (s)

Set property as variable
☐ Capture solution
☐ Contact time (s)
☐ Flow rate (µl/min)
☐ Extra wash solution

5.11.6 Inject and Recover

The example method provided for using the **InjectAndRecover** command is targeted to recovery for mass spectrometry, and contains two assay steps. The first conditions the surface by washing three times with 05% trifluoroacetic acid, while the second performs the sample injection and recovery operation.



1. Create assay steps by using the buttons below.

2. Select an assay step by clicking on it. Edit properties below or use buttons to remove or move the step.

Surface conditioning
[Conditioning] Conditioning 3 times as entered.

Inject and Recover
[Sample] Inject and Recover 1 time as entered.

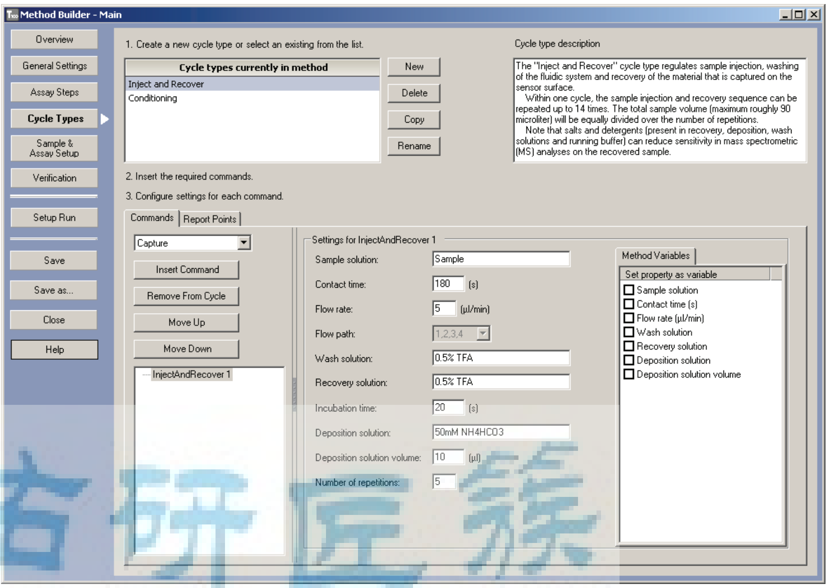
Base settings
Name: Surface conditioning
Purpose: Conditioning
Connect to cycle type: Conditioning

Recurrence
☐ Repeat assay step within
○ Every 1 cycle
○ Distribute 1 occurrences evenly
☐ Run assay step once first ☐ Run assay step once last

Number of replicates
3 times
● As entered (1,2,3,1,2,3)
○ Order (1,1,2,2,3,3)
○ Random

Assay step preparations
Temperature: 25
Buffer: A

The cycle for recovery of bound analyte contains just one single **InjectAndRecover** command. Additional commands are usually not needed, since the recovery component of the **InjectAndRecover** command serves as a regeneration step (see Section 5.6.1).



1. Create a new cycle type or select an existing from the list.

Cycle types currently in method
Inject and Recover
Conditioning

Cycle type description

The "Inject and Recover" cycle type regulates sample injection, washing of the fluidic system and recovery of the material that is captured on the sensor surface.

Within one cycle, the sample injection and recovery sequence can be repeated up to 14 times. The total sample volume (maximum roughly 90 microliter) will be equally divided over the number of repetitions.

Note that salts and detergents (present in recovery, deposition, wash solutions and running buffer) can reduce sensitivity in mass spectrometric (MS) analyses on the recovered sample.

2. Insert the required commands.

3. Configure settings for each command.

Capture

InjectAndRecover 1

Settings for InjectAndRecover 1

Sample solution: Sample
Contact time: 180 (s)
Flow rate: 5 (µl/min)
Flow path: 1,2,3,4
Wash solution: 0.5% TFA
Recovery solution: 0.5% TFA
Incubation time: 20 (s)
Deposition solution: 50mM NH4HCO3
Deposition solution volume: 10 (µl)
Number of repetitions: 5

Set property as variable
☐ Sample solution
☐ Contact time (s)
☐ Flow rate (µl/min)
☐ Wash solution
☐ Recovery solution
☐ Deposition solution
☐ Deposition solution volume

All parameters in the **InjectAndRecover** command are fixed: check the appropriate boxes in the **Method Variables** list if you want to use variable parameters. There are no evaluation variables for this command.

Evaluation Software

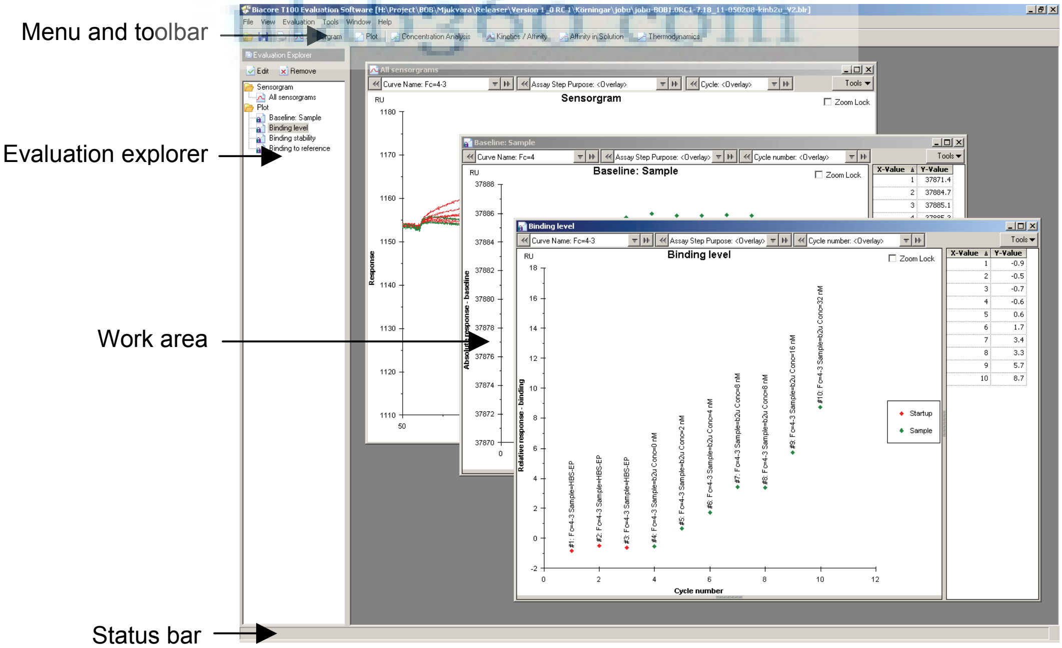
6. Evaluation software – general features

Biacore T100 Evaluation Software offers general functions for presentation of results as sensorgrams and/or report point plots and application-specific evaluation functions for concentration, kinetics and affinity, and thermodynamics measurement. There is also a function that corrects for solvent effects that can sometimes distort the results from analyses with low molecular weight analytes that give low response levels and require organic solvents (e.g. dimethyl sulfoxide, DMSO) to maintain solubility.

This chapter describes the organization of the evaluation software. The various evaluation functions are described in detail in the following chapters.

6.1 Evaluation software interface

The Biacore T100 Evaluation Software screen is divided into three main regions:



X-Value	Y-Value
1	37871.4
2	37884.7
3	37885.1

X-Value	Y-Value
1	-0.9
2	-0.5
3	-0.7
4	-0.6
5	0.6
6	1.7
7	3.4
8	3.3
9	5.7
10	8.7

- The ***menu and toolbar*** provide access to the evaluation functions.
- The ***Evaluation explorer*** at the left of the screen lists the evaluation items (plots, sensorgrams and other result displays) that have been created in the current session. Double-click on a folder in the evaluation explorer 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 that item.
- The ***work area*** displays the currently open items. Each item is shown in a separate window that can be moved, resized or closed independently of the other items.
- The ***status bar*** indicates the progress of current operations.

6.2 Opening files

To open a result file in the evaluation software, use the **File:Open** menu option. You can open result files from the control software (file extension .blr) and saved evaluation sessions (file extension .bme). If a file is already open in the software, opening a new file with **File:Open** will automatically close the first file.

Opening a file automatically creates a **Sensorgram** item and a number of default plots according to the content of the file.

To open multiple result files in the same session, use the **File:Append file** option. 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.

6.3 Common display functions

6.3.1 *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.

6.3.2 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. The list below describes the functions.

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

Displays the name of the evaluation item as a caption in the item window. The caption is shown as **Empty Plot** for sensorgram windows if there is no data to display.

Show sensorgram

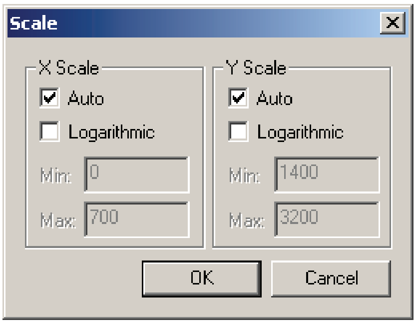
Displays the sensorgram relating to a point in the plot. This option is only available when you right-click on a point: the sensorgram is displayed in a separate window that must be closed before you can continue with the evaluation.

Exclude cycle/curve/point

Excludes data from the evaluation session. The data that can be excluded differs according to the type of window. Excluding cycles in either sensorgram or plot windows affects all other sensorgram and plot windows correspondingly: however, application-specific evaluation items that have already been created are not affected until they are edited and updated. If you have applied solvent correction and then exclude solvent correction cycles, the correction remains applied, but the excluded cycles will not be available if you edit the solvent correction item.

Scale

Sets the scale for the display. You can also access this function by double-clicking on either the x- or y-axis in the display.



X Scale
☑ Auto
☐ Logarithmic
Min: 0
Max: 700

Y Scale
☑ Auto
☐ Logarithmic
Min: 1400
Max: 3200

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, from where it can be pasted into third-party software such as word-processing or presentation programs.

Export curves
Exports 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.

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 box. The legend content varies with the type and settings for the display window, but in principle identifies the different curves or sets of points in the window.

6.4 Predefined evaluation items

When a result file is opened, a number of evaluation items are created automatically if the results contain the appropriate cycles and report points. This section describes the items created for all result files: special items are also created for certain wizard runs (see Chapter 4).

6.4.1 Sensorgram

An overlay plot of all sensorgrams is created and opened. The sensorgrams are not aligned, and are colored by assay step.

6.4.2 Plots

Plots are created for most wizards if the appropriate report point is present in the results. Separate plots will be created if there are multiple injections with similar report points (for example baseline for capture and sample injections). The **Plot Settings** cannot be changed, but the plot can be modified using the selectors and the **Tools** menu (see Section 7.2). Note that changing the selector settings can sometimes defeat the purpose of the plot.

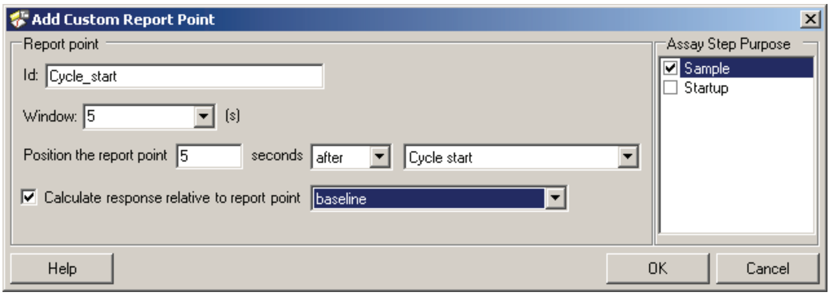
Baseline: Sample	Absolute response for report point **baseline** against cycle number.
Baseline: Capture	Absolute response for report point **capture_baseline** against cycle number.
Baseline: General	Absolute response for report point **general_baseline** against cycle number.
Binding to reference	Relative 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 (only for reference-subtracted curves).
Controls, binding	Relative response for the report point **binding** against cycle number for control samples (only for reference-subtracted curves).
Controls, stability	Relative response for the report point **stability** against cycle number for control samples.
Binding levels, binding	Relative response for the report point **binding** against cycle number for samples.
Binding levels, stability	Relative response for the report point **stability** against cycle number for samples (only for reference-subtracted curves).
Enhancement	Relative response for the report point **enhance_level** against cycle number for enhancement injections.

6.5 Custom report points

Report points are automatically created for all wizard- and method-based runs, placed at strategic positions in relation to injections. Choose **Tools:Custom Report Points** to add your own report points if the default report points are not sufficient for your purposes. You cannot edit the predefined report points.

Id	Position	Assay Step Purpose
Cycle_start	5 seconds after cycle start	Sample Control Sample

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



Id: Cycle_start

Window: 5 (s)

Position the report point 5 seconds after Cycle start

☑ Calculate response relative to report point baseline

☑ Sample
☐ Startup

Enter the id for the report point (maximum 30 characters) in the **Id** field. The report point id must be unique within an evaluation session.

Enter a value between 1 and 35 for the **Window**. The response value for the report point is the average response over the window, with the report time at the center of the window. A window of 5 seconds is recommended for most purposes.

Use the settings in the **Position the report point** field to define where the report point will be placed. Report points are placed a specified number of seconds before or after the beginning and end of injections or the beginning or end of the cycle.

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.

Check **Calculate response relative to report point** and select the required report point if you want to calculate the responses at the custom report point relative to another report point. If the box is not checked, the response will be calculated relative to the closest preceding baseline report point.

Check the assay step purpose(s) for which you want the report point to apply in the **Assay Step Purpose** list. Report point values are calculated for all cycles in the selected assay steps.

Editing and deleting report points

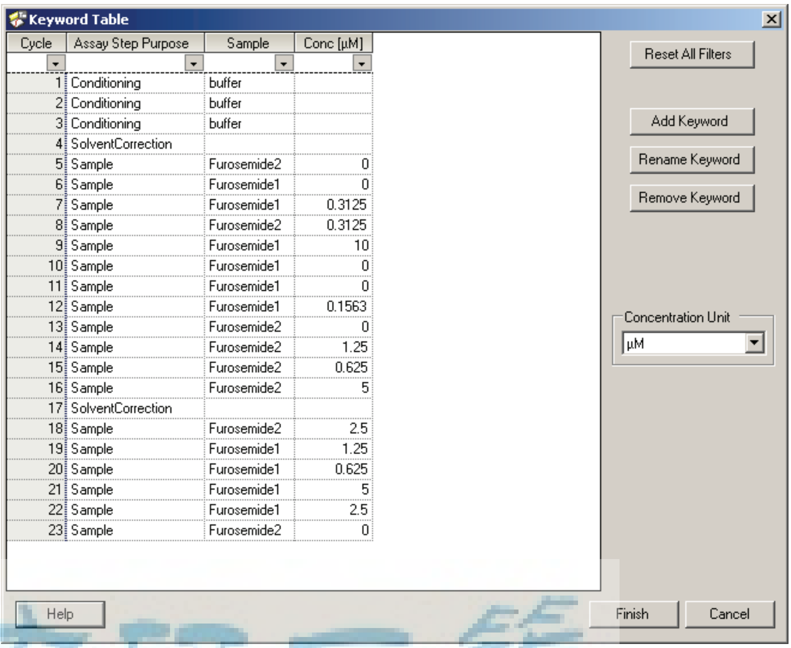
Select a report point in the list in the **Custom Report Points** dialog and click **Edit** to edit the report point definition or **Delete** to delete the report point. If you delete or change the name of a report point that has been used in a custom plot, the corresponding plot window will be empty.

6.6 Keywords

Keywords are assigned to cycles when the run is performed, and are then used for identification and evaluation purposes. Keywords are created automatically for wizard-based runs and may be defined in the method for method-based runs, and include:

- automatically generated identifiers such as cycle number or assay step purpose,
- method variables and predefined evaluation variables such as sample name, concentration and molecular weight,
- user-defined variables (see Section 5.6.2).

You can add and remove user-defined keywords in the evaluation software, and edit the contents of certain keywords. Choose **Tools:Keyword Table** to open the keyword table. When you edit the keyword table, all user-defined items in the evaluation session will be deleted. Save the session before editing the keyword table if you do not want to lose your work.



Cycle	Assay Step Purpose	Sample	Conc [µM]
1	Conditioning	buffer	
2	Conditioning	buffer	
3	Conditioning	buffer	
4	SolventCorrection		
5	Sample	Furosemide2	0
6	Sample	Furosemide1	0
7	Sample	Furosemide1	0.3125
8	Sample	Furosemide2	0.3125
9	Sample	Furosemide1	10
10	Sample	Furosemide1	0
11	Sample	Furosemide1	0
12	Sample	Furosemide1	0.1563
13	Sample	Furosemide2	0
14	Sample	Furosemide2	1.25
15	Sample	Furosemide2	0.625
16	Sample	Furosemide2	5
17	SolventCorrection		
18	Sample	Furosemide2	2.5
19	Sample	Furosemide1	1.25
20	Sample	Furosemide1	0.625
21	Sample	Furosemide1	5
22	Sample	Furosemide1	2.5
23	Sample	Furosemide2	0

Concentration Unit: µM

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

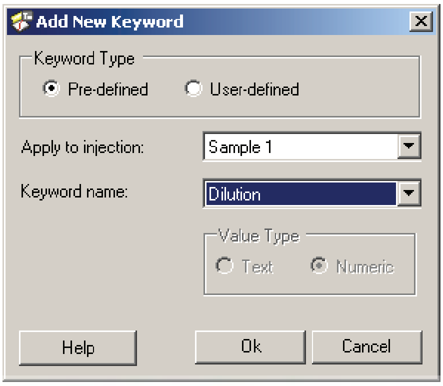
- Click on a column header to sort the table by the contents of that column.
- Click in the filter row (directly below the column header) and select a value to display only rows with that value for the chosen column. Click **Reset All Filters** to restore all filters to the **[All]** setting.

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 place the cursor in such a cell.

To change the units for concentration keywords, choose a new unit from the **Concentration Unit** list. The keyword values will be left unchanged but they will be interpreted for evaluation purposes in terms of the new unit. For example, a concentration entered as 10 µM will be evaluated as 10 mM if you change the concentration unit to mM. 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 the same for all files if data are to be evaluated together.

Note: The concentration unit affects only predefined concentration keywords. Numerical user-defined keywords are simply numbers, and will not be re-interpreted when you change the concentration unit even if they are intended to hold concentration information.

Click **Add Keyword** to create a new keyword in the table. You can choose between predefined keywords and user-defined keywords (see Section 5.6.2). If there are multiple **Sample** or **General** commands in the method from which the data is obtained, specify the command to which the new keyword should apply.



Keyword Type
● Pre-defined ○ User-defined

Apply to injection: Sample 1

Keyword name: Dilution

Value Type
○ Text ● Numeric

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

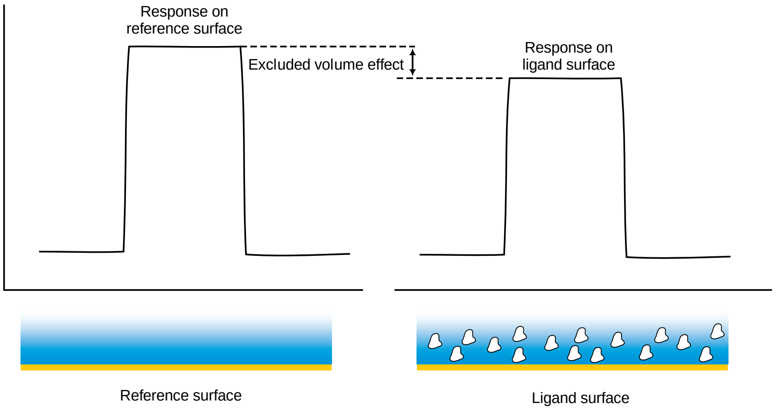
To rename or delete a keyword, click the appropriate button, then select the keyword in the dialog box. You cannot remove system-generated keywords such as file number or cycle number, or keywords derived from method variables such as sample name.

6.7 Solvent correction

6.7.1 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 solvents such as dimethyl sulfoxide (DMSO) to maintain solubility.

The need for solvent correction arises because subtraction of the reference response does not exactly eliminate the contribution of the bulk solution to the measured response. Bulk solution is excluded from the volume occupied by ligand on the active surface, so that the bulk contribution to the response on the active surface is slightly smaller than that on the reference surface (see illustration).



Bulk solution is excluded from the volume occupied by ligand molecules on the ligand surface, so the bulk contribution to the relative response is smaller than on the reference surface.

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.

Organic solvents like DMSO often give a high bulk response (addition of 1% DMSO 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 Biacore.

6.7.2 When solvent correction should be used

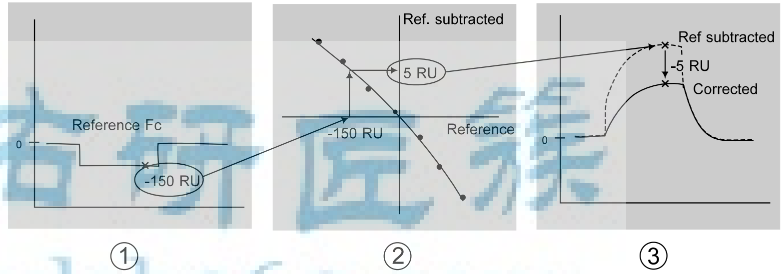
It is important to bear in mind that solvent correction is only relevant when

- the expected analyte responses are low (of the order of 50 RU or less),
- the ligand is a macromolecule immobilized at a high density (typically 5,000 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 measured 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.

6.7.3 How solvent correction works

Solvent correction factors are determined by injecting a series of blank samples containing a range of solvent concentrations, and plotting the difference in relative response between the active and reference surfaces as a function of 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 (see illustration).

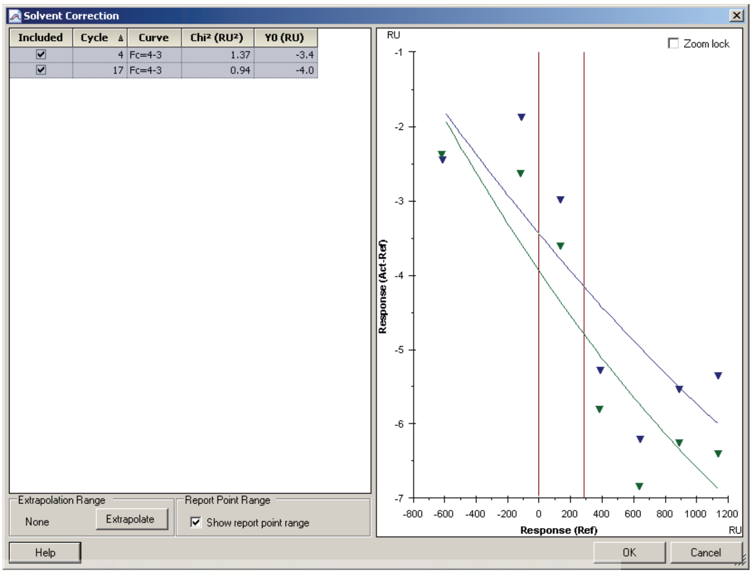


The principle of solvent correction. ***1.*** *The sensorgram from the reference flow cell shows a bulk displacement (-150 RU in the illustration) during sample injection because the sample and running buffer are not exactly matched.* ***2.*** *From the solvent correction curve, a displacement of –150 RU in the reference sensorgram corresponds to a solvent error of +5 RU in the reference-subtracted sensorgram.* ***3.*** *The reference-subtracted sensorgram is corrected by subtracting the solvent error. This procedure is applied to every 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. Before and after sample injection, when buffer is flowing over the surface, the bulk response is constant and correction is meaningless.

6.7.4 Applying solvent correction

To apply solvent correction, choose **Add solvent correction** from the **Evaluation** menu. In order to apply solvent correction, the run must include solvent correction cycles (see Section 5.6.1).



Included	Cycle	Curve	Chi² (RU²)	Y0 (RU)
☑	4	Fc=4-3	1.37	-3.4
☑	17	Fc=4-3	0.94	-4.0

Extrapolation Range: None

Report Point Range: ☑ Show report point range

☐ Zoom lock

The left-hand panel of this dialog lists the solvent correction curves in the run, and the corresponding solvent correction curves are shown in the right-hand panel. All cycles are shown by default in an overlay plot: click the rows in the cycle list (outside the **Included** check-box) to display specific cycles. Clear the checkmark in the **Included** box to exclude cycles from the correction calculation. You must include at least one solvent correction cycle for each curve. Sample responses are corrected according to the curve obtained from the nearest preceding correction cycle in the run. If there is no preceding correction cycle, the nearest following cycle is used.

Examine the curves for fitting to the experimental points. Right-click on outlying points to exclude either the single point from the curve fit or the whole correction cycle from the correction process. Statistical fitting parameters (chi-squared) are shown for each correction curve in the cycles list. Right-click on a point or curve in the right panel and choose **Show sensorgrams** if you want to examine the sensorgrams from solvent correction cycles as an aid in judging the quality of the data.

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. If the range of solvent correction is small (as in the illustration above), points may appear to scatter widely from the fitted curve.

The solvent correction curve is fitted to the experimental points using a second-degree equation. Beware of applying solvent correction if the correction curve does not fit the experimental points closely. Scatter in the correction points indicates that the measurements are not reliable, and applying correction derived from such curves can distort the measured responses unnecessarily.

The range of report point values that are candidates for solvent correction in the assay data is indicated by vertical red lines in the window. If report points lie outside the correction range, these values cannot be properly corrected. Some *small* extrapolation of the correction plots may be permissible. Use the **Extrapolate** button to extend the correction range. The shape of the solvent correction plots is however not fully predictable, and extrapolation over more than a short distance (at most 10% of the range of the reference values) is dangerous.

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.

7. Data presentation tools

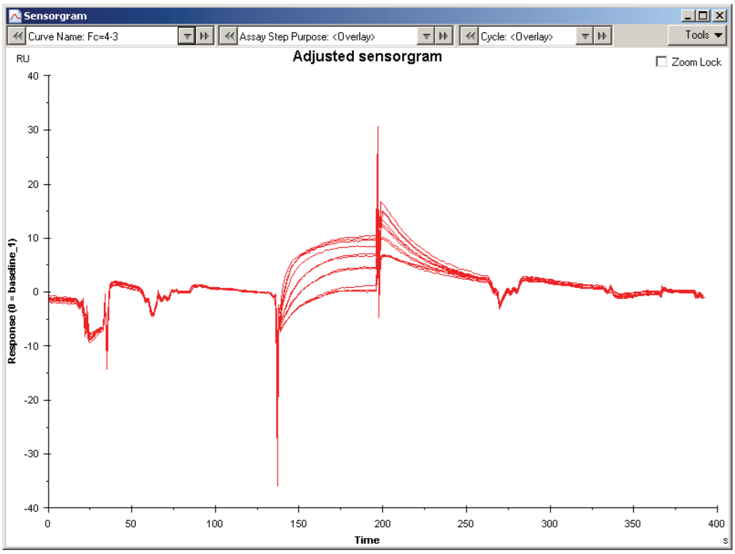
This chapter describes the tools available for presentation and examination of the data in a result set. These tools comprise:

- Sensorgram display, with facilities for aligning sensorgrams in overlay plots.
- Plot tools for displaying and ranking response values.
- A report point table for listing numerical values associated with report points.

7.1 Sensorgram windows

Sensorgram windows display the sensorgrams from one or more cycles in the result set.

To create sensorgram display window, click **Sensorgram** in the toolbar or choose **Add sensorgram** from the **Evaluation** menu. The window is created directly with an overlay plot of all cycles in the result set. You can open any number of sensorgram windows with independent display settings. The window is listed in the evaluation explorer as **Sensorgram *n***, where ***n*** is a serial number. Right-click on the sensorgram item in the evaluation explorer and choose **Rename** to change the item name.



Curve Name: Fc=4-3

Assay Step Purpose: <Overlay>

Cycle: <Overlay>

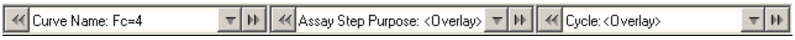
☐ Zoom Lock

Hold the cursor over a sensorgram to display a tool tip identifying that particular curve. The sensorgram coloring can be changed if desired with the **Tools:Color by** option.

The following sections describe display functions specific to sensorgram windows. General display functions are described in Section 6.3.

7.1.1 Selecting sensorgrams for display

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



Curve Name: Fc=4

Assay Step Purpose: <Overlay>

Cycle: <Overlay>

- **Curve name** lists the type of sensorgram (active, reference, reference subtracted and solvent corrected where applicable).

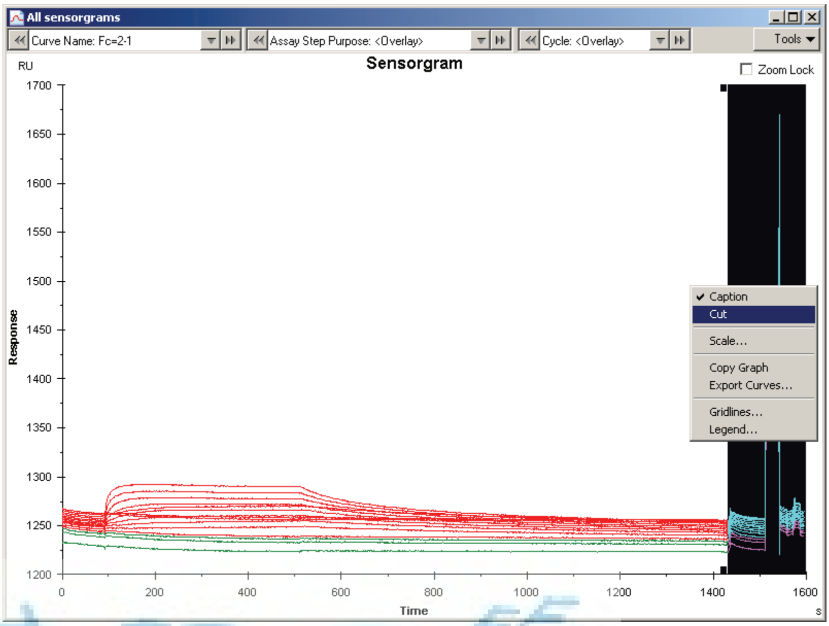
- **Assay Step Purpose** filters the sensorgram according to the assay step purpose.

- **Cycle** lists all the cycles in the result set. When multiple files are open, cycles are identified with two numbers, one for the file in the result set and one for the cycle within the file (thus cycle 1-10 is the 10th cycle in the first file added to the result set; cycle 2-4 is the 4th cycle in the second file and so on).

For each option, 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 to select contiguous multiple items. Use control-click to select non-contiguous multiple items. To accept a selection, click anywhere outside the list or press Enter.

7.1.2 Removing data

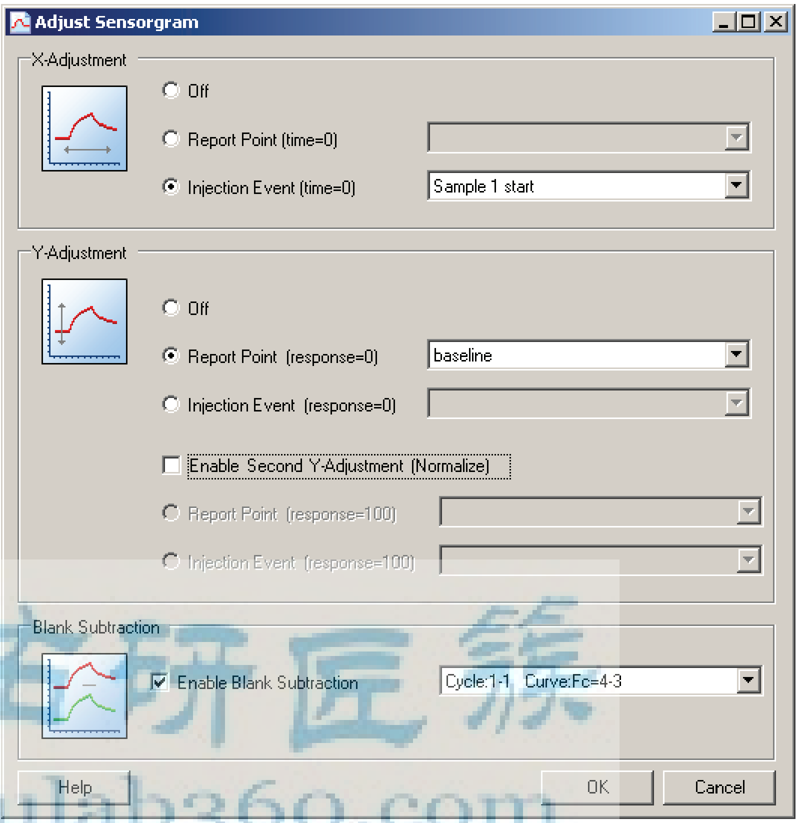
To remove data from the display, mark the section to be removed by dragging with the right mouse button, then choose **Cut** from the right-click menu. The data will be removed from the current sensorgram window only; no other windows or evaluation items will be affected.



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

7.1.3 Sensorgram adjustment

Choose **Sensorgram adjustment** under the **Tools** button for options for aligning and adjusting the sensorgram display. For curve alignment, sensorgrams which do not include the chosen reference point for alignment will not be shown.



Adjust Sensorgram

X-Adjustment

○ Off

○ Report Point (time=0)

● Injection Event (time=0) Sample 1 start

Y-Adjustment

○ Off

● Report Point (response=0) baseline

○ Injection Event (response=0)

☐ Enable Second Y-Adjustment (Normalize)

○ Report Point (response=100)

○ Injection Event (response=100)

Blank Subtraction

☑ Enable Blank Subtraction Cycle:1-1 Curve:Fc=4-3

X-adjustment

Choose 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 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.

If you check **Enable Second Y-Adjustment**, you can select a report point or injection event where the response value will be set to 100. Each sensorgram will then be normalized separately to the first and second adjustment point, so that all sensorgrams will have values of 0 and 100 at these points regardless of the original response levels. 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).

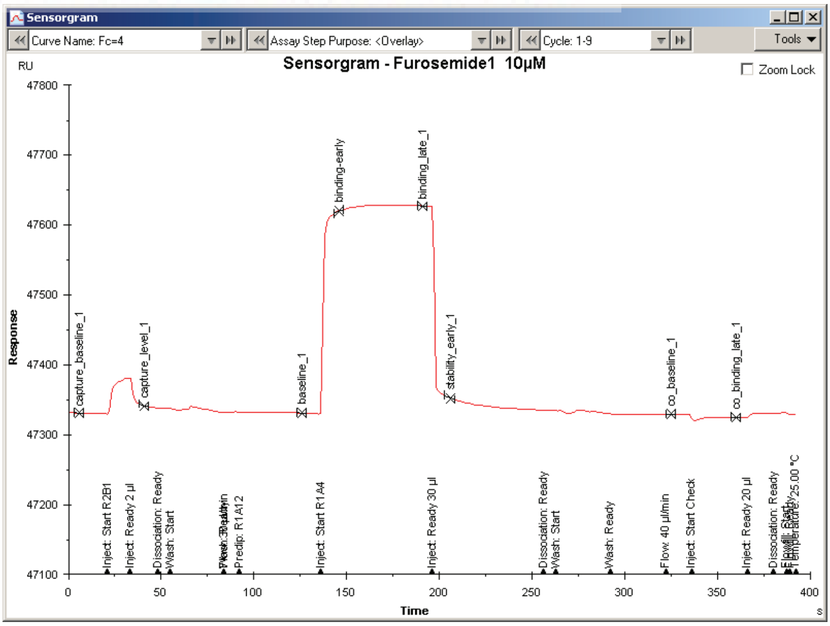
Blank subtraction
Check **Enable Blank Subtraction** and choose a curve to be used as the blank to subtract one sensorgram from all others in the display. Use this feature to eliminate systematic disturbances in sensorgrams that are not removed by reference subtraction. Blank subtraction only affects the current sensorgram window: other evaluation items are not affected.

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 curve from all others in the result set.

To cut data from the sensorgram display, drag with the right mouse button to select the data you want to remove and then choose **Cut** from the right-click menu. This can be useful to eliminate e.g. regeneration pulses from the sensorgram display. Cutting data affects only the current sensorgram window.

7.1.4 Markers

You can choose to display markers and/or labels for report points and events in the cycle with 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.

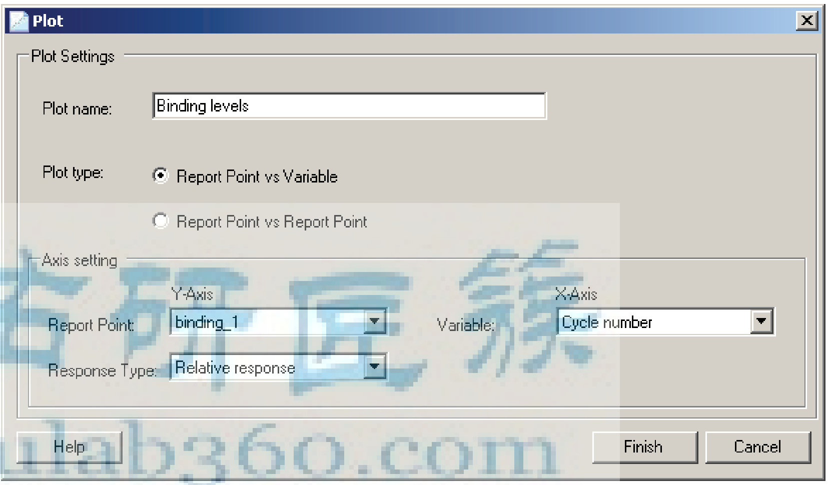


Sensorgram display with labels and markers shown for both report points and events.

7.2 Plot windows

Plot windows display report point values plotted against either variables or other report point values in the same cycle. Curves can be fitted to points using linear or 4-parameter fitting functions. Ranking borders can be added to plot windows to classify response levels into groups such as high, medium and low responses.

To create a plot window, click **Plot** in the toolbar or choose **Add plot** from the **Evaluation** menu. Enter a name for the plot, choose the parameters that define the plot and click **Finish**.



Plot name: Binding levels

Plot type: ● Report Point vs Variable ○ Report Point vs Report Point

Y-Axis

Report Point: binding_1

Response Type: Relative response

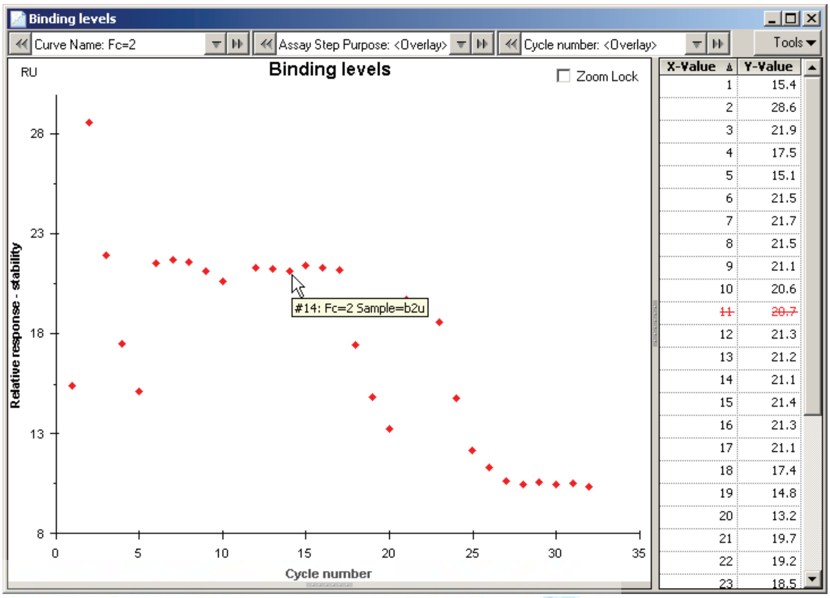
X-Axis

Variable: Cycle number

Response values may be absolute or relative response or slope, or relative response adjusted for molecular weight if the keyword **MW** is defined. Adjustment for molecular weight is performed by dividing the response in RU by the molecular weight in Da. Points for which the molecular weight value is zero or missing are omitted from the plot. Cycles that do not contain the selected report point will not be represented in the plot.

Variables may be numerical (e.g. molecular weight or concentration) or non-numerical (e.g. sample name or assay step purpose).

The plot will be created with default display settings, with a graphical representation at the left and a table of x-and y-values 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).

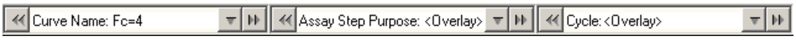


X-Value	Y-Value
1	15.4
2	28.6
3	21.9
4	17.5
5	15.1
6	21.5
7	21.7
8	21.5
9	21.1
10	20.6
~~11~~	~~20.7~~
12	21.3
13	21.2
14	21.1
15	21.4
16	21.3
17	21.1
18	17.4
19	14.8
20	13.2
21	19.7
22	19.2
23	18.5

Right-click on the plot item in the evaluation explorer and choose **Rename** to change the plot name.

7.2.1 Selector functions

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



- **Curve name** lists the type of sensorgram from which the points are taken (active, reference, reference subtracted and solvent corrected where applicable).
- **Assay Step Purpose** filters the points according to the assay step purpose.
- The third selector lists the variable values represented on the x-axis. (This option is not available for plots of report point against report point.)

Selection operates in the same way as in the sensorgram window (Section **Error! Reference source not found.**).

Other general display functions are described in Section 6.3.

7.2.2 Table functions

The table to the right of the plot area lists values for the points in the plot. Excluded points are shown struck out in red text. You can 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.

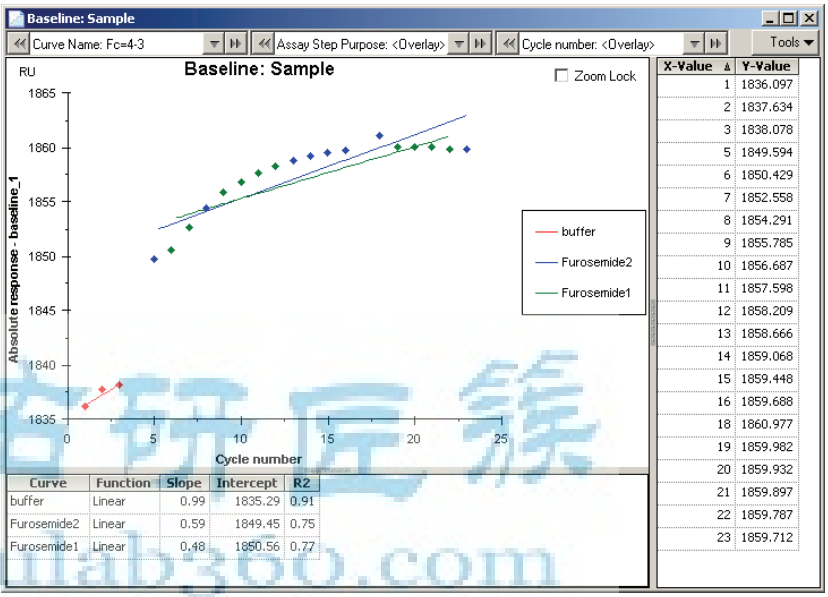
By default, the table shows x- and y-values and is sorted in ascending order of x-values. Click on the header row to select the sort value and to change the sort order. Sorting the table does not have any effect on the plot display.

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).

☑ X-Value
☑ Y-Value
☑ Ranking
☐ Cycle number
☐ CycleType
☑ AssayStep
☐ AssayStepPurpose
☐ Temp
☐ Sample
☐ Fc

7.2.3 Fitting curves to points

Choose **Curve Fitting** under the **Tools** button to fit lines to the points in the plot. You can choose linear or curved (4-parameter) fitting functions. The numerical fitting results are displayed in an extra panel below the plot. 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.



Curve	Function	Slope	Intercept	R2
buffer	Linear	0.99	1835.29	0.91
Furosemide2	Linear	0.59	1849.45	0.75
Furosemide1	Linear	0.48	1850.56	0.77

X-Value	Y-Value
1	1836.097
2	1837.634
3	1838.078
5	1849.594
6	1850.429
7	1852.558
8	1854.291
9	1855.785
10	1856.687
11	1857.598
12	1858.209
13	1858.666
14	1859.068
15	1859.448
16	1859.688
18	1860.977
19	1859.982
20	1859.932
21	1859.897
22	1859.787
23	1859.712

For linear fitting, the points are fitted to the equation

$$\mathbf{y} = \text{slope} * \mathbf{x} + \text{intercept}$$

The equation for a 4-parameter fit is

$$\mathbf{y} = R_{hi} - \frac{R_{hi} - R_{lo}}{1 + \left(\frac{\mathbf{x}}{A_1}\right)^{A_2}}$$

where **y** and **x** are the plot coordinates, R_{hi} and R_{lo} are fitting parameters that correspond to the maximum and minimum response levels respectively, and A_1 and A_2 are additional fitting parameters.

The closeness of fit is reported for linear fitting as the correlation coefficient R^2 and for 4-parameter fitting as the chi-squared value.

Choose **Curve parameters** under the **Tools** button to toggle display of the fitting parameters below the plot panel.

7.2.4 Ranking

Choose **Ranking** under the **Tools** button to add ranking boundaries to the plot. You can add one or two boundaries, classifying the plot points as Low/High or Low/Medium/High. The boundary values are specified in RU.

Ranking Settings

○ None

○ One Ranking Boundary

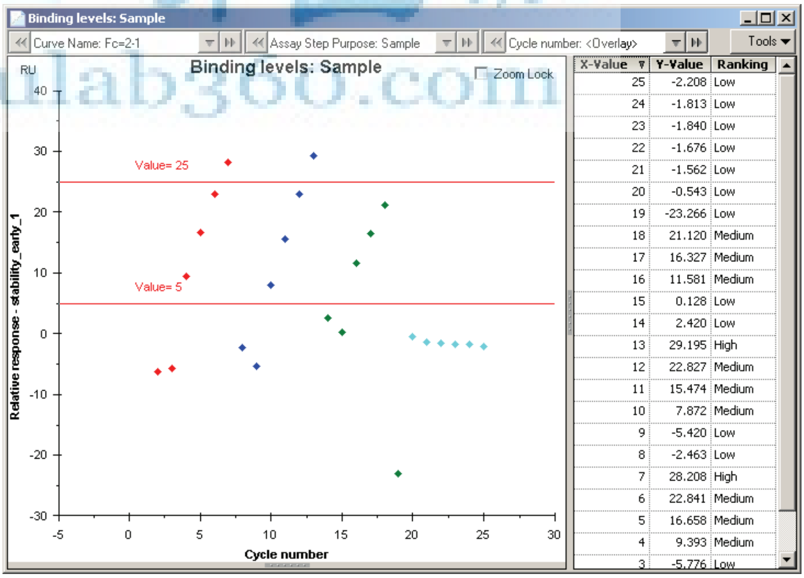
● Two Ranking Boundaries

Ranking Boundaries

First Value: 5

Second Value: 25

Boundaries are shown as horizontal red lines in the plot, labeled with the boundary value. The classification of the points is recorded in the table.



X-Value	Y-Value	Ranking
25	-2.208	Low
24	-1.813	Low
23	-1.840	Low
22	-1.676	Low
21	-1.562	Low
20	-0.543	Low
19	-23.266	Low
18	21.120	Medium
17	16.327	Medium
16	11.581	Medium
15	0.128	Low
14	2.420	Low
13	29.195	High
12	22.827	Medium
11	15.474	Medium
10	7.872	Medium
9	-5.420	Low
8	-2.463	Low
7	28.208	High
6	22.841	Medium
5	16.658	Medium
4	9.393	Medium
3	-5.776	Low

Ranking results are independent for each plot window.

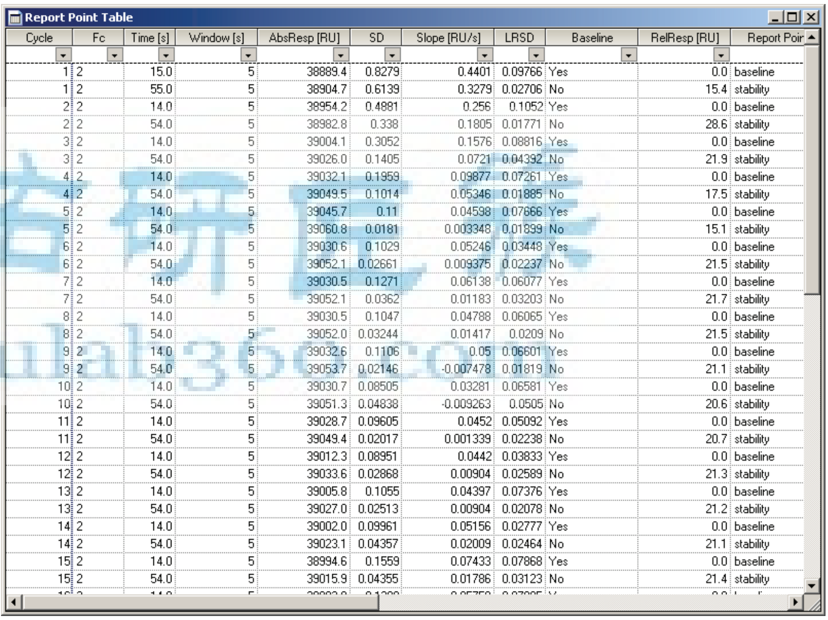
Note: Editing the definition of a plot does not affect ranking boundaries. If you for example change the y-axis parameter of a ranked plot from relative response to absolute response, you will need to revise the placing of the ranking boundaries if the ranking is to remain meaningful.

7.3 Report point table

Report points are automatically created for all wizard- and method-based runs, placed at strategic positions in relation to injections. See Section 6.5 for details of how to add custom report points.

7.3.1 Displaying the report point table

The report point table lists numerical values for all report points in the current result set. Choose **Evaluation:Add Report Point Table** to create a report point table window. You can only add one report point table window to each evaluation session: the table is updated automatically if you add custom report points or apply solvent correction.



Report Point Table

Cycle	Fc	Time [s]	Window [s]	AbsResp [RU]	SD	Slope [RU/s]	LRSD	Baseline	RelResp [RU]	Report Poir
1	2	15.0	5	38889.4	0.8279	0.4401	0.09766	Yes	0.0	baseline
1	2	55.0	5	38904.7	0.6139	0.3279	0.02706	No	15.4	stability
2	2	14.0	5	38954.2	0.4881	0.256	0.1052	Yes	0.0	baseline
2	2	54.0	5	38982.8	0.338	0.1805	0.01771	No	28.6	stability
3	2	14.0	5	39004.1	0.3052	0.1576	0.08816	Yes	0.0	baseline
3	2	54.0	5	39026.0	0.1405	0.0721	0.04392	No	21.9	stability
4	2	14.0	5	39032.1	0.1959	0.09877	0.07261	Yes	0.0	baseline
4	2	54.0	5	39049.5	0.1014	0.05346	0.01885	No	17.5	stability
5	2	14.0	5	39045.7	0.11	0.04598	0.07666	Yes	0.0	baseline
5	2	54.0	5	39060.8	0.0181	0.003348	0.01899	No	15.1	stability
6	2	14.0	5	39030.6	0.1029	0.05246	0.03448	Yes	0.0	baseline
6	2	54.0	5	39052.1	0.02661	0.009375	0.02237	No	21.5	stability
7	2	14.0	5	39030.5	0.1271	0.06138	0.06077	Yes	0.0	baseline
7	2	54.0	5	39052.1	0.0362	0.01183	0.03203	No	21.7	stability
8	2	14.0	5	39030.5	0.1047	0.04788	0.06065	Yes	0.0	baseline
8	2	54.0	5	39052.0	0.03244	0.01417	0.0209	No	21.5	stability
9	2	14.0	5	39032.6	0.1106	0.05	0.06601	Yes	0.0	baseline
9	2	54.0	5	39053.7	0.02146	-0.007478	0.01819	No	21.1	stability
10	2	14.0	5	39030.7	0.08505	0.03281	0.06581	Yes	0.0	baseline
10	2	54.0	5	39051.3	0.04838	-0.009263	0.0505	No	20.6	stability
11	2	14.0	5	39028.7	0.09605	0.0452	0.05092	Yes	0.0	baseline
11	2	54.0	5	39049.4	0.02017	0.001339	0.02238	No	20.7	stability
12	2	14.0	5	39012.3	0.08951	0.0442	0.03833	Yes	0.0	baseline
12	2	54.0	5	39033.6	0.02868	0.00904	0.02589	No	21.3	stability
13	2	14.0	5	39005.8	0.1055	0.04397	0.07376	Yes	0.0	baseline
13	2	54.0	5	39027.0	0.02513	0.00904	0.02078	No	21.2	stability
14	2	14.0	5	39002.0	0.09961	0.05156	0.02777	Yes	0.0	baseline
14	2	54.0	5	39023.1	0.04357	0.02009	0.02464	No	21.1	stability
15	2	14.0	5	38994.6	0.1559	0.07433	0.07868	Yes	0.0	baseline
15	2	54.0	5	39015.9	0.04355	0.01786	0.03123	No	21.4	stability

The contents of the report point table cannot be edited.

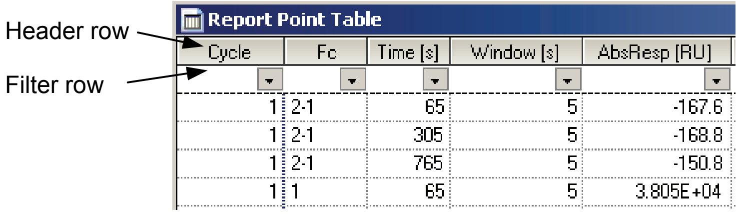
The following columns are represented in the report point table:

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.
Cycle	Cycle number within the file.
Fc	The curve to which the report point applies, identified as the flow cell.

Time	Report point time in seconds from the start of the cycle.
Window	Report point time window in seconds.
AbsResp	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 $\mathrm{SD} = \sqrt{\frac{1}{(n-1)}\sum(y_i - \bar{y})^2}$ where n = number of points and y = response in RU
Slope	Slope during time window in RU s^{-1}, calculated as $\mathrm{slope} = \frac{\sum\left((y_i - \bar{y})^2 (x_i - \bar{x})\right)}{\sum(x_i - \bar{x})^2}$
LRSD	Alignment of slope to a straight line (regression coefficient), calculated as $\mathrm{LRSD} = \sqrt{\frac{Q_0}{(n-2)}}$ where $Q_0 = \sum(y_i - \bar{y})^2 - \frac{\left(\sum(y_i - \bar{y})(x_i - \bar{x})\right)^2}{\sum(x_i - \bar{x})^2}$
Baseline	**Yes** for report points defined as baseline. Otherwise **No.**
RelResp	Relative response (difference between AbsResp and baseline) in RU. #N/A if no baseline has been set.
Report point	Report point id.
CycleType **AssayStep** **AssayStep Purpose**	Identifiers for the cycles, set explicitly in methods (see Chapter 5) and automatically in wizards.
Keywords	One column is created for each keyword in the data.

Sorting and filtering the report point table

The report point table can be sorted by any column in ascending or descending order for any column. Click in the header for a column to sort the table by that column. Each click in the same header toggles the sort order.



Cycle	Fc	Time [s]	Window [s]	AbsResp [RU]
1	2-1	65	5	-167.6
1	2-1	305	5	-168.8
1	2-1	765	5	-150.8
1	1	65	5	3.805E+04

The second row in the report point table contains a filter setting for each column. All values will be included if the filter setting is blank. To apply a filter, click on the setting and select 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 report point table contents

To copy the 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. The selected 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, including header cells and filter settings.

8. Concentration analysis

Biacore T100 software supports evaluation of concentration analysis using both linear and non-linear calibration curves determined by measurement of known samples.

8.1 Requirements for concentration evaluation

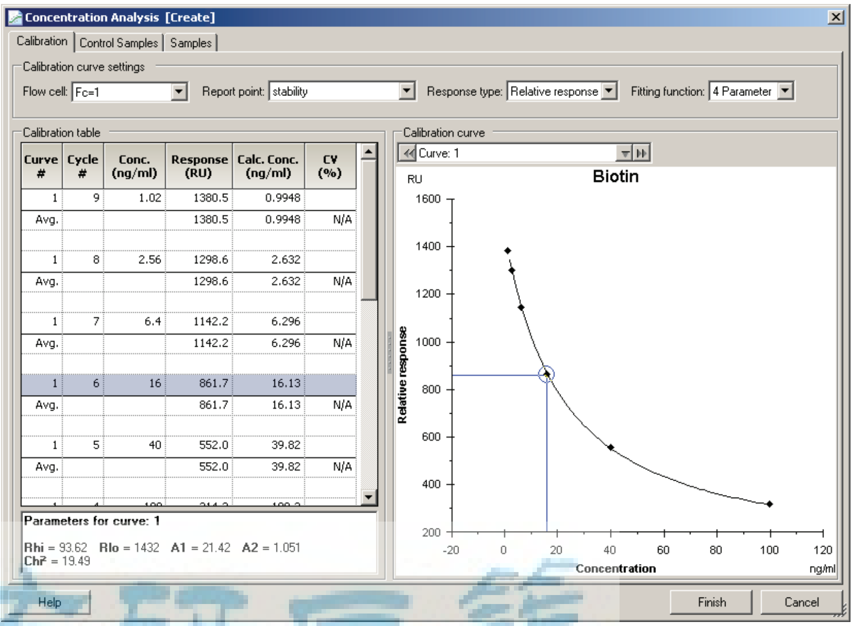
In order to evaluate concentration analysis from a Biacore T100 run, the run must include at least one calibration curve and unknown sample injections must have the appropriate properties and keywords. For wizard-based runs, the conditions are met automatically when the **Concentration Analysis** wizard is used. For method-based runs, the method must be constructed as described in Section 5.10.1: if necessary, the keyword table can be edited so that the conditions are met in full (see Section 6.6). Note however that the command name cannot be edited in the keyword table. Refer to Chapter 5 for details of how to construct methods in Method Builder.

Evaluation of unknown samples is based on the nearest preceding calibration curve. If there is no preceding calibration curve in the run, the nearest following curve is used.

8.2 Evaluating concentration analysis

To evaluate a concentration analysis run, open the run and click **Concentration Analysis** in the evaluation toolbar, or choose **Add Concentration Analysis** from the **Evaluation** menu. The evaluation dialog is divided into three tabs, for calibration curves, control samples and unknown samples respectively. Choose the appropriate settings for the calibration curves and click **Finish** to complete the evaluation. There are no settings that can be changed for control samples or unknown samples.

8.2.1 Calibration curves



Calibration curve settings

Flow cell: Fc=1
Report point: stability
Response type: Relative response
Fitting function: 4 Parameter

Calibration table

Curve #	Cycle #	Conc. (ng/ml)	Response (RU)	Calc. Conc. (ng/ml)	CV (%)
1	9	1.02	1380.5	0.9948	
Avg.			1380.5	0.9948	N/A
1	8	2.56	1298.6	2.632	
Avg.			1298.6	2.632	N/A
1	7	6.4	1142.2	6.296	
Avg.			1142.2	6.296	N/A
1	6	16	861.7	16.13	
Avg.			861.7	16.13	N/A
1	5	40	552.0	39.82	
Avg.			552.0	39.82	N/A

Parameters for curve: 1

Rhi = 93.62 Rlo = 1432 A1 = 21.42 A2 = 1.051
Chi^2 = 19.49

A calibration curve is constructed from the cycles in each calibration step. If two calibration steps are run in direct succession so that there are no other cycles between the steps, they will be treated as a single calibration curve.

Settings on the **Calibration** tab define the report point and fitting function that are used to create the calibration curve. The settings apply to all calibration curves in the evaluation. Choose the settings appropriate for your analysis:

Flow cell	Concentration analysis may be performed without a reference cell, since the unknown samples are determined by direct reference to a calibration curve obtained under the same conditions. If a reference cell is included in the flow path for the run, the reference-subtracted curve may however be selected if desired.
Report point	Response levels for concentration analysis (relative response) are normally taken from a report point shortly after the end of the sample injection, to avoid contributions from the bulk refractive index of the sample. For analyses based on the rate of binding (report point slope), a report point early in the sample injection is normally used.
Response type	Choose between relative response and slope.

Fitting function Choose between linear and 4-parameter functions. The 4-parameter function is a general fitting function for continuous curves, and is recommended for most purposes. Use a linear function only if you have good reason to expect the calibration curve to be a straight line. See Section 7.2.3 for the equations for fitting functions.

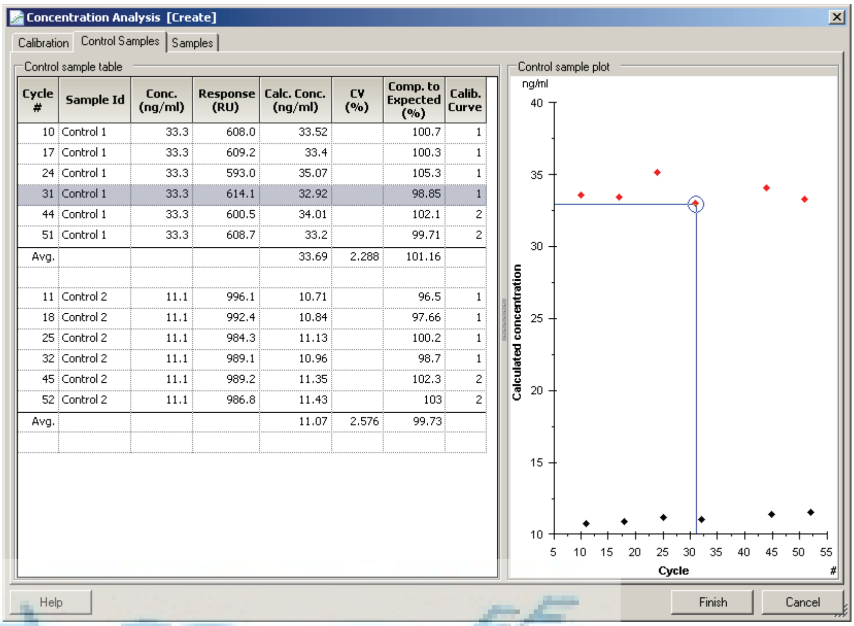
The left-hand panel lists the details of the calibration curve data, with concentration and response or slope values for all calibration points. The **Calc conc** column lists the concentration corresponding to the actual response value as determined from the fitted calibration curve. Select a row in the table to highlight the corresponding point on the calibration curve.

The calibration curves are shown in the right-hand panel. Choose the curve to display from the list at the top of the panel. Each calibration curve is constructed from the cycles in an assay step with the purpose **Calibration**. Right-click on a curve or calibration point for options for excluding calibration curves or single cycles from the evaluation. Excluded cycles are shown as open symbols and excluded curves are shown as broken lines. The excluded points are marked in the left-hand panel with red strikethrough text. You can also choose to display sensorgrams for individual calibration cycles from the right-click menu.

Note: The sample name is ignored when the calibration curve is constructed. For wizard-based runs, the sample name will be the same for all calibration cycles, but different names can be introduced either in method-based runs or by editing the keyword table. Changing sample names in calibration cycles has no effect on the calibration curve.

8.2.2 Control samples

The **Control Samples** tab displays the measured concentration for control samples as a trend plot of response against cycle number.



Cycle #	Sample Id	Conc. (ng/ml)	Response (RU)	Calc. Conc. (ng/ml)	CV (%)	Comp. to Expected (%)	Calib. Curve
10	Control 1	33.3	608.0	33.52		100.7	1
17	Control 1	33.3	609.2	33.4		100.3	1
24	Control 1	33.3	593.0	35.07		105.3	1
31	Control 1	33.3	614.1	32.92		98.85	1
44	Control 1	33.3	600.5	34.01		102.1	2
51	Control 1	33.3	608.7	33.2		99.71	2
Avg.				33.69	2.288	101.16	
11	Control 2	11.1	996.1	10.71		96.5	1
18	Control 2	11.1	992.4	10.84		97.66	1
25	Control 2	11.1	984.3	11.13		100.2	1
32	Control 2	11.1	989.1	10.96		98.7	1
45	Control 2	11.1	989.2	11.35		102.3	2
52	Control 2	11.1	986.8	11.43		103	2
Avg.				11.07	2.576	99.73	

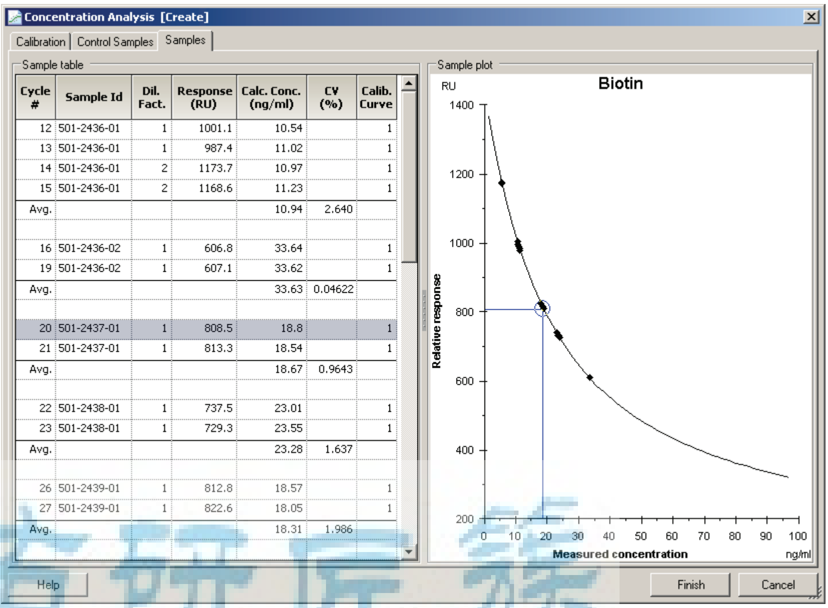
Control sample concentrations are calculated from the nearest preceding calibration curve (or the nearest following if there is no preceding curve). Numerical results are presented in the table at the left, and plotted as calculated concentration against cycle number on the right. Select a row in the table to highlight the corresponding point on the plot.

The table lists the expected concentration as entered for the control samples, the response and calculated concentration, the calculated concentration as a percentage of expected and the calibration curve used to calculate the concentration. Replicate control samples are summarized with average values and coefficient of variation (CV%) for the response and calculated concentration.

Right-click on a sample row in the table or a sample point on the curve and choose **Exclude Cycle** to exclude that sample from the sample evaluation. Excluded cycles are shown as open symbols and are marked in the table with red strikethrough text.

8.2.3 Samples

The **Samples** tab displays the measured concentrations for samples.



Cycle #	Sample Id	Dil. Fact.	Response (RU)	Calc. Conc. (ng/ml)	CV (%)	Calib. Curve
12	501-2436-01	1	1001.1	10.54		1
13	501-2436-01	1	987.4	11.02		1
14	501-2436-01	2	1173.7	10.97		1
15	501-2436-01	2	1168.6	11.23		1
Avg.				10.94	2.640	
16	501-2436-02	1	606.8	33.64		1
19	501-2436-02	1	607.1	33.62		1
Avg.				33.63	0.04622	
20	501-2437-01	1	808.5	18.8		1
21	501-2437-01	1	813.3	18.54		1
Avg.				18.67	0.9643	
22	501-2438-01	1	737.5	23.01		1
23	501-2438-01	1	729.3	23.55		1
Avg.				23.28	1.637	
26	501-2439-01	1	812.8	18.57		1
27	501-2439-01	1	822.6	18.05		1
Avg.				18.31	1.986	

Sample concentrations are calculated from the nearest preceding calibration curve (or the nearest following if there is no preceding curve). The left-hand panel lists the results sorted by sample ID, with averages and CV values for each sample ID. The column **Calc.Conc.** gives the concentration calculated for the original sample, obtained as the measured concentration multiplied by the dilution factor. Concentrations for samples that give a response outside the range of the calibration curve are listed as N/A (not applicable). Select a row in the table to highlight the corresponding point on the plot.

The right-hand panel shows the calibration curve and samples calculated from it for the currently selected sample. Sample points represent the measured concentrations, not the values calculated for the original samples.

Right-click on a sample row in the table or a sample point on the curve and choose **Exclude Cycle** to exclude that sample from the average sample calculation. Excluded cycles are shown as open symbols and are marked in the table with red strikethrough text.

8.2.4 Evaluating combined result sets

If you use the **Append File** function to combine result sets from separate runs, concentration analysis can be evaluated provided that the conditions specified in Section 5.10 are fulfilled in the combined set. The software does not check the validity of any evaluation applied to a combined result set, so that it is your responsibility to determine that the evaluation results are meaningful. It is for example in principle possible to append a kinetic analysis result file to a concentration analysis, and then apply concentration analysis evaluation: provided that the report point used for the calibration curve exists in the cycles from the kinetic run, calculated concentrations will be reported for these samples.

In order to ensure that concentration analysis is correctly evaluated in combined result sets, make sure that all files that contribute to the combined result set are derived from concentration analysis runs. Provided that each file starts with a calibration curve that is not excluded from the evaluation, the results will be calculated within the respective files even in the combined result set. However, if calibration curves at the beginning of files are missing or excluded, there will be overlap between the individual file sets and some samples from one file will be evaluated on the basis of calibration curves from another file. In such cases, it is important to ensure that the calibration curves and sample analyses in the different files refer to the same analyte and are performed under as far as possible identical condition.

9. Kinetics and affinity analysis

Biacore T100 offers three main functions for analysis of interaction kinetics and affinity:

- Kinetics and affinity measurements on the sensor surface, which determine the interaction characteristics between ligand and analyte. Kinetic parameters are evaluated from the association and dissociation phases of the sensorgram, and affinity either from the kinetic parameters or from plots of steady-state analyte binding levels (R_{eq}) against concentration.
- Thermodynamic analysis, which relies on measurement of either kinetics or affinity at different temperatures.
- Affinity in solution, where the interactants are mixed in known concentrations in solution and allowed to reach equilibrium. Biacore T100 is then used to determine the concentration of free interactant in equilibrium with the complex.

This chapter describes how to evaluate surface-bound kinetics and affinity. Thermodynamic analysis and measurement of affinity in solution are described in Chapters 10 and 11 respectively.

9.1 Kinetics and affinity determination

9.1.1 Requirements for kinetics and affinity evaluation

The minimum requirements for evaluation of kinetics or affinity are one cycle with a **Sample** injection in an assay step with purpose **Sample**, with a sample concentration in the keyword **Conc**. If the concentration is not given in molar units, the keyword **MW** must also be included with a value for the molecular weight. For method-based runs, the method must be correctly constructed as described in Section 5.10.2: if necessary, the keyword table can be edited so that the conditions are met in full (see Section 6.6). Note however that the command name cannot be edited in the keyword table. Refer to Chapter 5 for details of how to construct methods in Method Builder.

The recommended minimum conditions for kinetic and affinity evaluation are

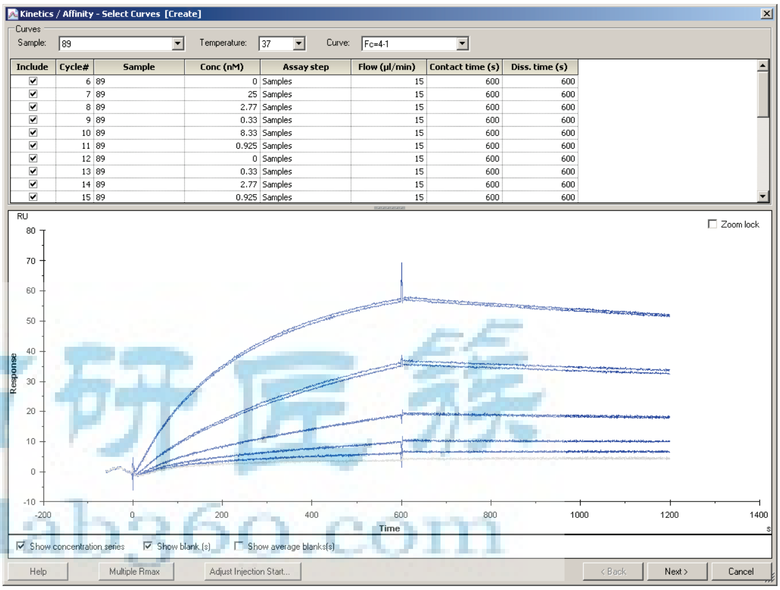
- a concentration series of analyte with at least five non-zero concentrations and one zero concentration
- duplicate determinations for at least one non-zero concentration.

These conditions are recommended but not mandatory in the **Kinetics/Affinity** wizard.

9.1.2 Evaluating kinetics and affinity

To start a kinetics or affinity evaluation, click the **Kinetics/Affinity** button on the toolbar or choose **Add Kinetics/Affinity** from the **Evaluation** menu.

1. The first dialog presents the concentration series available in the current result set and allows you to choose the curves included in the evaluation.



Kinetics / Affinity - Select Curves [Create]

Curves

Sample: 89 Temperature: 37 Curve: Fc=4-1

Include	Cycle#	Sample	Conc (nM)	Assay step	Flow (µl/min)	Contact time (s)	Diss. time (s)
☑	6	89	0	Samples	15	600	600
☑	7	89	25	Samples	15	600	600
☑	8	89	2.77	Samples	15	600	600
☑	9	89	0.33	Samples	15	600	600
☑	10	89	8.33	Samples	15	600	600
☑	11	89	0.925	Samples	15	600	600
☑	12	89	0	Samples	15	600	600
☑	13	89	0.33	Samples	15	600	600
☑	14	89	2.77	Samples	15	600	600
☑	15	89	0.925	Samples	15	600	600

☐ Zoom lock

☑ Show concentration series ☑ Show blank (s) ☐ Show average blanks(s)

A concentration series is defined by a set of curves with the same sample name, analysis temperature and curve identity. Select the concentration series you want to work with in the respective pull-down lists.

Note: Sample names are case-sensitive, so that “Sample” and “sample” belong to different concentration series. Edit the sample names in the keyword table if you have unintentionally mixed upper- and lower-case letters.

If the result set contains data from more than one file, curves with the same sample name, temperature and curve identity are grouped together in a single concentration series.

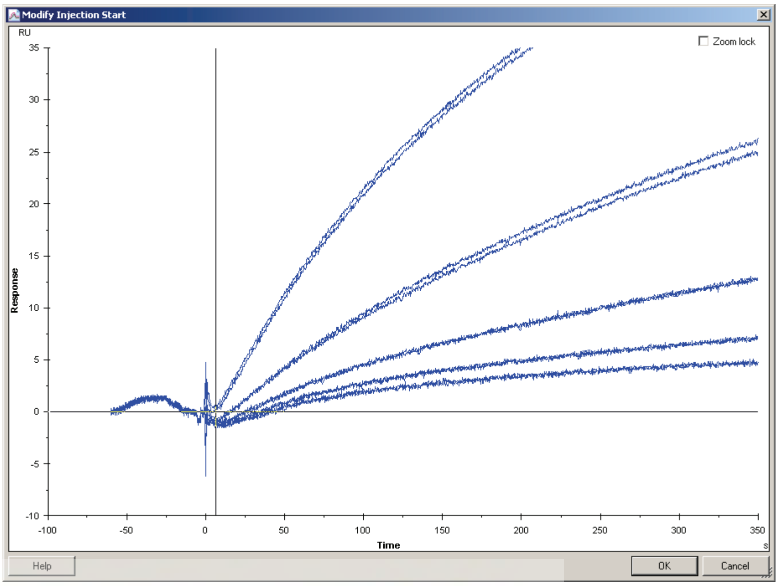
Use the **Include** column in the table of curves to choose which curves should be included in the data set to be evaluated. You can select several curves and use the right-click menu to exclude or include multiple curves in one operation. By default, all curves for the sample are included: sensorgrams for non-zero concentrations are shown in color, and those for blanks (zero concentrations) in light gray. The sensorgrams are adjusted to zero at the start of the sample injection on both the response and time axes. The average of the blank sensorgrams will be automatically subtracted from the other curves when you proceed to the next step. If you do not want to perform blank subtraction, exclude the zero concentration sensorgrams from the data set. You can also choose to use blanks from other concentration series for blank subtraction: these are listed at the bottom of the table, and are excluded by default. Only blank sensorgrams with the same contact and dissociation times as the samples are used.

For runs that use captured ligand, the ligand is listed in the table. Make sure you only include curves that are derived from the same ligand.

The three check-boxes below the sensorgram panel control the type of curves shown in the display. You can use these check-boxes to examine the sample and blank curves without interference from each other, and to show the average blank that will be used for subtraction. Bear in mind however that these boxes control the display only and do not affect the data set that will be evaluated.

If you have multiple ligand densities represented in the result set, click **Multiple Rmax** to assign curves to the different sets (see Section 9.1.3).

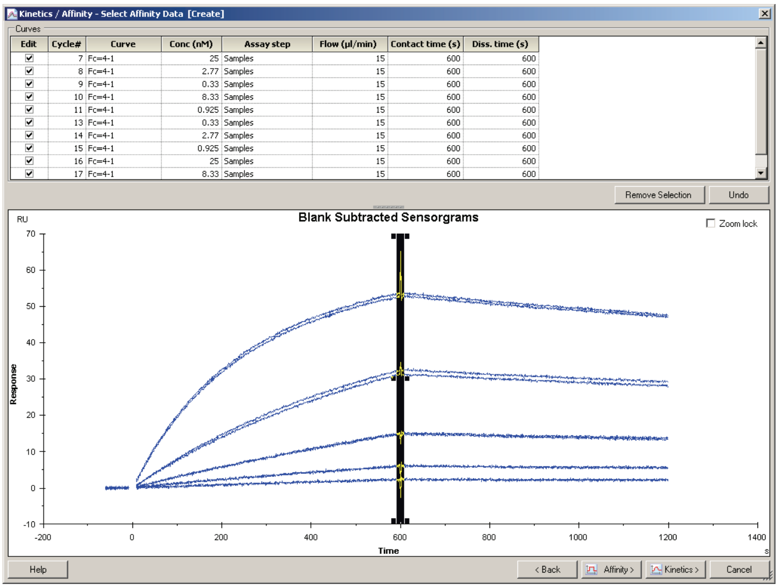
Click **Adjust Injection Start** if the sample injections do not start at precisely zero time. The injection start is set automatically from the event marker, but may need slight adjustment for best fitting results. The adjustment compensates for small systematic discrepancies in the interval between the start of the injection as recorded in the event log and the time that the same actually reaches the sensor surface. The difference is most apparent at low flow rates. The discrepancy has very little effect on the rate constants obtained from the fitting except for very fast reactions, and adjusting the injection start is seldom necessary.



Drag the vertical reference line to adjust the injection start point. You can adjust the start by ±10 s from zero. The same adjustment is applied to all curves in the data set, whether they are currently included for evaluation or not.

Click **Next>** when you have selected the curves to be evaluated.

2. The second dialog shows the blank subtracted curve set and allows you to delete selected regions from all or selected curves, for example to eliminate spikes or other disturbances.



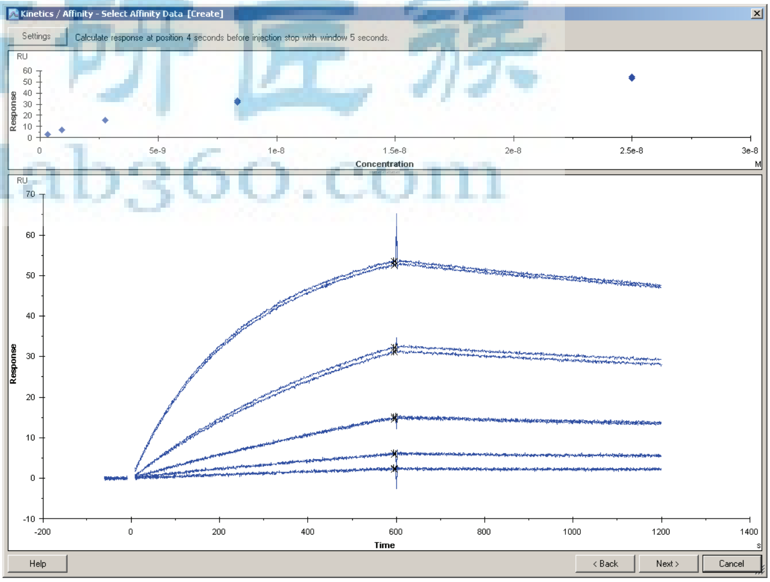
Edit	Cycle#	Curve	Conc (nM)	Assay step	Flow (µl/min)	Contact time (s)	Diss. time (s)
☑	7	Fc=4-1	25	Samples	15	600	600
☑	8	Fc=4-1	2.77	Samples	15	600	600
☑	9	Fc=4-1	0.33	Samples	15	600	600
☑	10	Fc=4-1	8.33	Samples	15	600	600
☑	11	Fc=4-1	0.925	Samples	15	600	600
☑	13	Fc=4-1	0.33	Samples	15	600	600
☑	14	Fc=4-1	2.77	Samples	15	600	600
☑	15	Fc=4-1	0.925	Samples	15	600	600
☑	16	Fc=4-1	25	Samples	15	600	600
☑	17	Fc=4-1	8.33	Samples	15	600	600

To delete a selected region from all curves, drag with the right mouse button over the region to be deleted and click **Remove Selection**. Click **Undo** to restore the deleted data.

If you want to delete a region from only selected curves, remove the checkmark from the **Edit** column in the 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 color. Note that 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.

Click **Affinity** for steady state affinity evaluation or **Kinetics** for kinetics evaluation when you are satisfied with the curves.

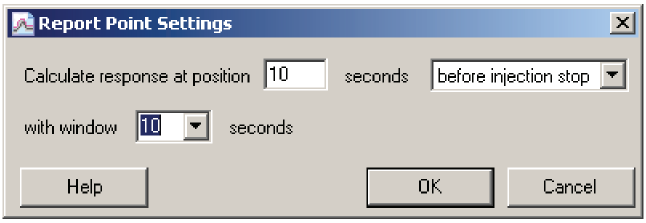
3. (*Affinity only*). If you choose to evaluate steady state affinity, the next dialog gives a preview of the plot of steady state response against concentration, with the option to adjust the selection of data used to calculate response values.



Calculate response at position 4 seconds before injection stop with window 5 seconds.

Note: The data set illustrated here is not in fact suitable for affinity analysis. Sensorgrams should approach steady state at all concentrations for reliable determination of affinity constants.

The top panel shows the plot of R_{eq} against C, based on average response values over the region marked on the sensorgrams. Click **Settings** to adjust the region used for calculation of R_{eq} values.



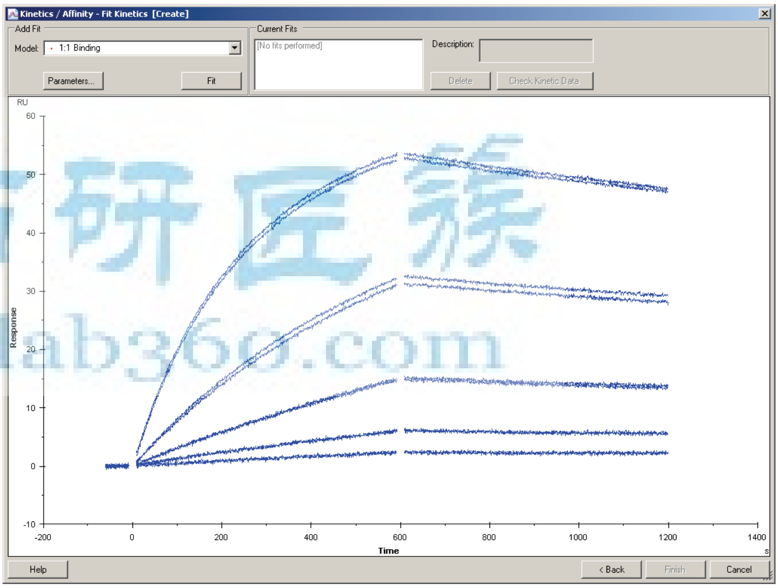
Report Point Settings

Calculate response at position 10 seconds before injection stop

with window 10 seconds

Click **Next>** when you are satisfied with the data selection.

4. In the next dialog (applicable to both kinetics and affinity), you select the fitting model and perform the fit. The same data can be fitted repeatedly to different models or to the same model with different settings.



Kinetics / Affinity - Fit Kinetics [Create]

Model: 1:1 Binding

[No fits performed]

Select the model from the pull-down list. Available models are described in Section 9.3. Click **Parameters** if you want to change the starting values or scope of any of the parameters (see Section 9.2.2 for details), then click **Fit** to perform the fitting.

During the fitting procedure, the fitted curves are shown in black overlaid on the experimental data. Fitting progress is indicated in the sensorgram window by display of the iteration number, the current chi-squared value and the relative change in the parameter that was changed most from the previous iteration. You can use the **Abort** or **Accept** buttons to cancel the fitting or accept the fitting after the current iteration. You may want to cancel the fitting if it is clear that a fit cannot be found, or to accept the fitting if the chi-squared value and/or maximum relative change indicate that an acceptable fit has been achieved. Clicking on

Accept will stop the fitting at the end of the current iteration, which may take a few moments to complete.

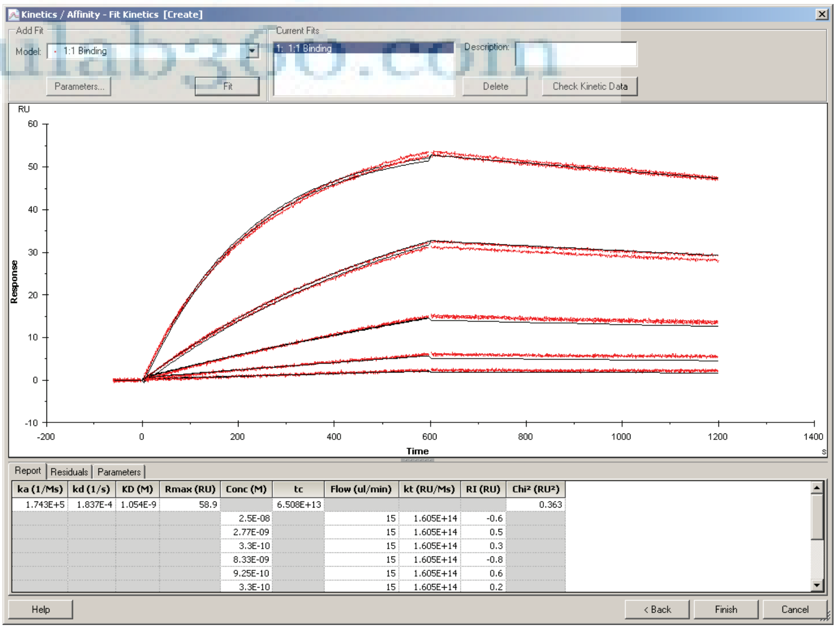
You can enter a short description for the fit in the **Description** box. This can be useful for example to distinguish different fits for thermodynamic analysis (Section 10.2).

To perform additional fits on the same data, choose a new model or new parameter settings and click **Fit**. To remove a fit from the evaluation item, select the fit in the list of current fits and click **Delete**.

Click **Finish** to complete the evaluation and place the item in the evaluation explorer panel. You can click **Back** to review the choice of data for the evaluation: however, if you make any changes to the data (e.g. remove additional sections from a curve or exclude a curve from the set), all current fits will be deleted. Current fits are also deleted if you switch between kinetics and affinity evaluation.

Kinetics results

When the fit is completed, the results for kinetics are displayed as fitted curves overlaid in black on the experimental data, with details in the table below the curves:



Model: 1:1 Binding

1: 1:1 Binding

Description:

ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Conc (M)	tc	Flow (ul/min)	kt (RU/Ms)	RI (RU)	Chi² (RU²)
1.743E+5	1.837E-4	1.054E-9	58.9		6.508E+13				0.363
				2.5E-08		15	1.605E+14	-0.6	
				2.77E-09		15	1.605E+14	0.5	
				3.3E-10		15	1.605E+14	0.3	
				8.33E-09		15	1.605E+14	-0.8	
				9.25E-10		15	1.605E+14	0.6	
				3.3E-10		15	1.605E+14	0.2	

The detailed results are presented on three tabs:

- **Report** shows selected parameters and calculated values. The contents of the **Report** tab are defined in the model. Global parameters are listed on a single row at the top of the table, and local parameters are listed on one row for each curve.

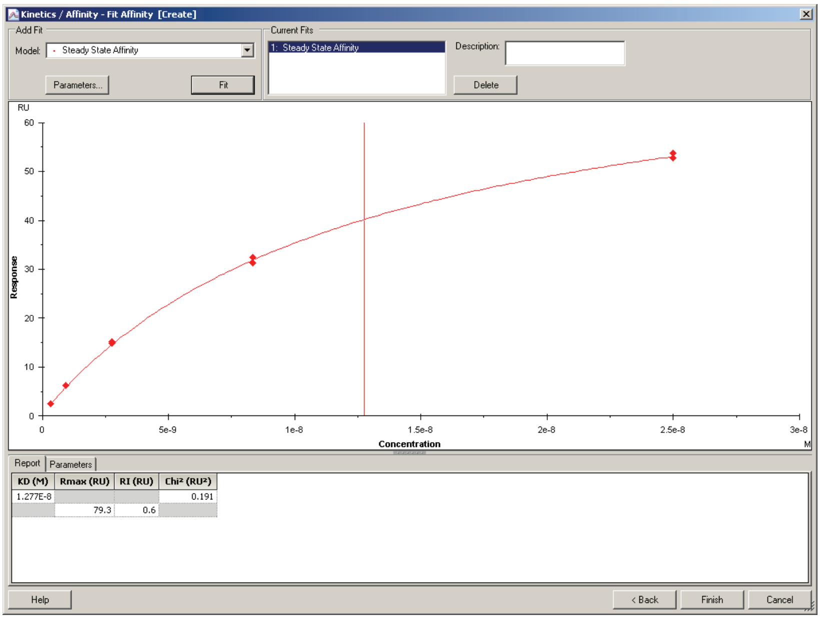
- **Residuals** plots the difference between the experimental and fitted curves for each point in the curves. Use this display as an aid in judging how closely the results fit the experimental data.

- **Parameters** shows the values for all parameters in the fitting equations.

Kinetic constants obtained from the fitting procedure are only significant if the observed binding is not seriously limited by mass transport of analyte to the surface (see Section 9.3). For 1:1 fitting results, you can check whether mass transport is limiting or not using the **Check Kinetic Data** function as described in Section 9.2.3.

Affinity results

For affinity determination, the reported K_D value is marked on the plot as a vertical line (for a 1:1 interaction, K_D is the same as the analyte concentration at a response equal to half R_{max}). If the reported value is higher than half the highest concentration used, this line will be shown broken in red as a warning that the value may be unreliable because the plot does not flatten out sufficiently.



Model: Steady State Affinity

Current Fits: 1: Steady State Affinity

Description:

KD (M)	Rmax (RU)	RI (RU)	Chi² (RU²)
1.277E-8			0.191
	79.3	0.6	

The detailed results are presented on two tabs:

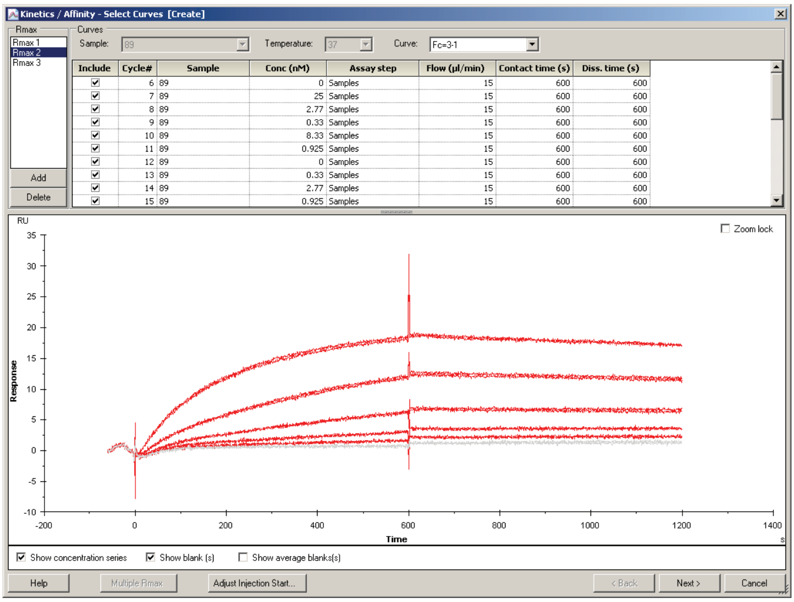
- **Report** shows selected parameters and calculated values. The contents of the **Report** tab are defined in the model. Global parameters are listed on a single row at the top of the table, and local parameters are listed on one row for each curve.

- **Parameters** shows the values for all parameters in the fitting equations.

9.1.3 Multiple ligand densities

Analysis of the same analyte concentration series over multiple ligand densities can provide more robust fitting than a single ligand density. The kinetic and steady-state affinity fitting functions support simultaneous analysis of up to five sets of data with independent values for R_{max}, returning a single set of rate constants for the whole combined data set. Analyses over multiple ligand densities may be performed in separate runs that are combined with the **Append file** function, but should preferably be performed on multiple flow cells in the same run to ensure that the experimental conditions are comparable as far as possible.

To set up evaluation of the same sample series over multiple ligand densities, click **Multiple Rmax** in the first dialog box for kinetics and affinity evaluation. A panel for data subsets representing different R_{max} values opens to the left of the curve table.



Sample: 89 Temperature: 37 Curve: Fc=3-1

Include	Cycle#	Sample	Conc (nM)	Assay step	Flow (µl/min)	Contact time (s)	Diss. time (s)
☑	6	89	0	Samples	15	600	600
☑	7	89	25	Samples	15	600	600
☑	8	89	2.77	Samples	15	600	600
☑	9	89	0.33	Samples	15	600	600
☑	10	89	8.33	Samples	15	600	600
☑	11	89	0.925	Samples	15	600	600
☑	12	89	0	Samples	15	600	600
☑	13	89	0.33	Samples	15	600	600
☑	14	89	2.77	Samples	15	600	600
☑	15	89	0.925	Samples	15	600	600

☑ Show concentration series ☑ Show blank (s) ☐ Show average blanks(s)

Click **Add** to add a new data subset. If you have multiple sets of the currently chosen curve type in the evaluation session (e.g. multiple reference subtracted curves), the next curve in the list is assigned to the new data subset. If there are no more curves of the same type available, the new set will be a copy of the most recently created subset. You can add up to five data subsets, representing five ligand densities. The same sample name and analysis temperature apply to all subsets.

Click on a subset to manage the contents of the subset. You cannot mix subsets that use different samples or different temperatures.

Caution: Make sure that the same curve numbers are not assigned to more than one data subset. If a curve is assigned to two data sets, the software will try to evaluate the same curve with two different Rmax values, and the fitting may be distorted.

Do not evaluate multiple copies of the same subset for multiple ligand densities. Subsets that are duplicated will be weighted more than those that are not duplicated in the fitting procedure.

Do not attempt to use subsets for any purpose other than multiple ligand densities. The data will be evaluated in terms of multiple ligand densities regardless of how you have assigned curves to the subsets.

The subsets will be evaluated together, with rate constants that are global for the whole data set and Rmax values that are global within each subset but can differ between subsets.

Note: Evaluation with a global Rmax parameter within subsets is achieved by using a control parameter ***in*** which is set to 1 for the *n*th subset and 0 for all others. Rmax is then fitted as Rmax*i*n*, which returns a non-zero value for subset *n* only. These ***in*** parameters are listed on the **Parameters** tab in the results.

9.2 Curve fitting principles

With all kinetic and affinity analysis, it is important to remember that the results obtained represent the results of fitting the experimental data to a mathematical model, and that 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.

9.2.1 Fitting procedure

Kinetic parameters are extracted from experimental data by an iterative process that finds the best fit for a set of equations describing the interaction. The equations are created automatically from the definition of the interaction model. The fitting process begins with initial values for the parameters in the equation set, and optimizes the parameter values according to an algorithm that minimizes the chi-squared value for the fitting. Chi-squared is a measure of the average squared residual (the difference between the experimental data and the fitted curve):

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

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

For sensorgram data, the number of data points is very much larger than the number of fitted parameters in the model, so

$$n - p \approx n$$

and chi-squared reduces to the average squared residual per data point. If the model fits the experimental data precisely, chi-squared represents the mean square of the signal noise.

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. On occasion, however, if can be necessary to adjust the starting values for fitting parameters, accessed through the **Parameters** button in the fitting dialog (step 4 in Section 9.1.2 above).

9.2.2 Local and global parameters

Parameters in the fitting equations are treated as either ***local*** or ***global variables*** or ***constants***:

- Local parameters are assigned an independent value for each curve in the data set. 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 parameters have one single value that applies to the whole data set. Typical global parameters are the rate constants for the interaction, which should in principle have the same value for all curves in the data set.

- 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).

The local/global status of parameters can be changed through the **Parameters** button in the fitting dialog (step 4 in Section 9.1.2 above), without making any changes to the model.

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, kinetic 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 (e.g. in a capture assay, if the capture level varies between cycles).

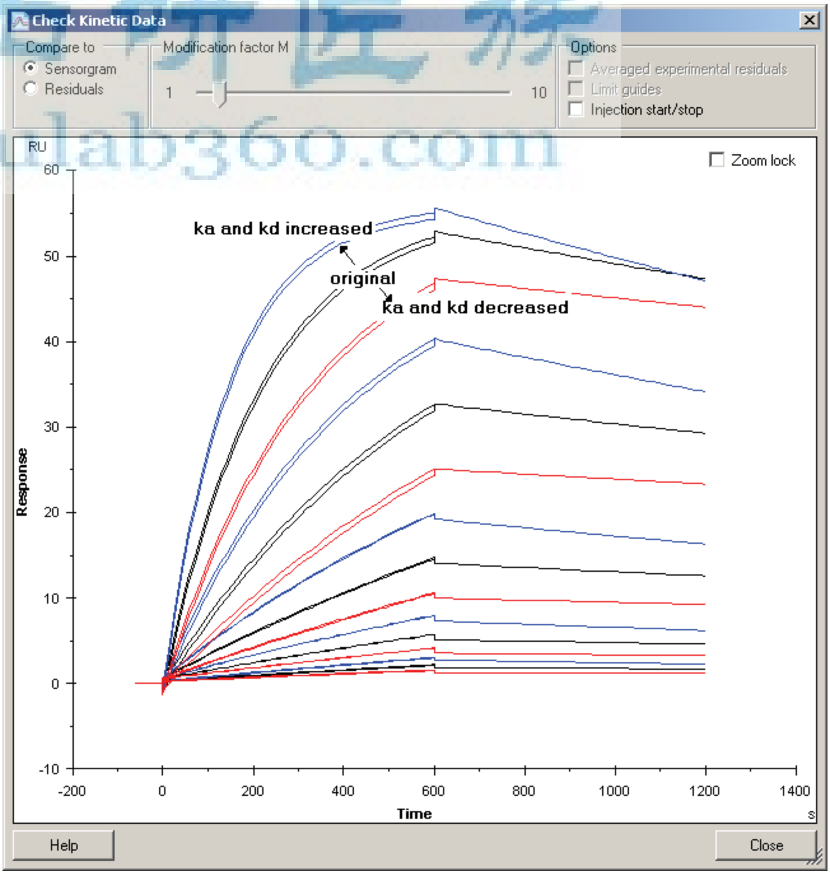
9.2.3 Parameter significance

The evaluation procedure necessarily returns values for all parameters in the fitting equations, even if some parameters may not always be relevant for a particular data set. The software provides two tools for determining the significance of reported parameters, **Check Kinetic Data** and the standard error (SE) or T-value.

Check Kinetic Data

Clicking on the **Check Kinetic Data** opens a dialog 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, 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. 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.

This tool is only available for results obtained with the 1:1 fitting model.



● Sensorgram
○ Residuals

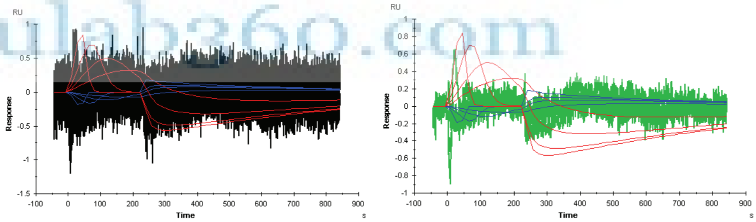
1 — 10

☐ Averaged experimental residuals
☐ Limit guides
☐ Injection start/stop

☐ Zoom lock

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 (reduced rate constants) and blue (increased rate constants) 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 because the binding is fully limited by mass transfer. Mass transfer 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 examine the effect of varying the modification factor on the difference between the original and modified curves in relation to the experimental residuals. The tool display allows the simulated difference curves to be compared to the experimental residuals or to residuals averaged over a moving time window. The latter option smooths the experimental residual display, making it easier to observe the general shape of the residual curves. Movable horizontal **Limit guides** can be displayed to mark the extent of the residual variation and aid visual interpretation. (Note that the limit guides do not in any way imply acceptance limits.)



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

Note: To use the **Check Kinetic Data** tool on a finished kinetic evaluation item, open the item for editing and step forward to the last stage of the kinetic evaluation.

Standard error or T-value

The significance of parameter values is indicated by the standard error (SE) or T-value listed on the **Parameters** tab in the fitting results. This is a statistical indication of the significance of a fitted parameter. Lower standard error values indicate higher significance: if the standard error represents less than 10% of the parameter value, the parameter is significant for the experimental data.

For ease of comparison between parameters with widely different absolute values (e.g. k_a and k_d), the standard error may be expressed as a ***T-value***, which is obtained by dividing the value of the parameter by the standard error. A high T-value corresponds to a low standard error. As a general guideline, parameters with a T-value greater than about 10 should be regarded as significant.

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.

The significance of a parameter is a measure of how much a change in the parameter value affects the closeness of fit. A parameter with low significance can have a wide range of values without affecting the fit. 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 $RU{\cdot}M^{-1}s^{-1}$, but evaluation of data with no mass transfer limitation might return a value of 10^{12} or higher. Similarly, rate constants that lack significance are often assigned values outside the reasonable range for biomolecular interactions, or outside the range that can be measured with Biacore.

Notes: The standard error and the **Check Kinetic Data** tool assess parameter significance in different ways, even if the results of the assessment may sometimes be related. **Check Kinetic Data** tests the contribution of a group of parameters (rate constants for the interaction and mass transport processes) to the closeness of fit by examining the results of correlated changes, whereas the standard error is a mathematical assessment of the significance of a single parameter. If the **Check Kinetic Data** tool indicates that the rate constants are not significant, the standard error for the constants may be expected to be high. However, the converse is not always true (a high standard error will not always be reflected in the behavior of the **Check Kinetic Data** tool).

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 for all parameters. Consistency of a value between repeated evaluations is ***not*** a test of significance.

9.3 Predefined models

A set of predefined models for kinetics and steady state affinity is provided with Biacore T100 Evaluation Software. These models are marked in the model selection list (see Section 9.1.2) with a red dot, and cannot be removed or modified.

Mass transfer parameters

All kinetic models include a term for mass transfer of analyte to the surface. If transport 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 (see Section 9.2.3).

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 $m \cdot s^{-1}$):

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

where D is the diffusion coefficient of the analyte
f is the volume flow rate of solution through the flow cell
h, w, l are the flow cell dimensions (height, width, length)

One form used in fitting models in Biacore T100 is referred to as the ***mass transfer constant*** k_t (units $RU \cdot M^{-1} \cdot m \cdot s^{-1}$), obtained by adjusting the mass transfer coefficient approximately for the molecular weight of the analyte and for the conversion of surface concentration to RU:

$$k_t = k_m \times MW \times 10^9$$

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

$$tc = \frac{k_t}{f^{1/3}}$$

9.3.1 Kinetics – 1:1 binding

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 are

		Obtained from
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)	Provided as input
tc	Flow rate-independent component of the mass transfer constant	Fitted
f	Flow rate (µl/min)	Provided as input
tOn	Sample injection start time (s)	Provided as input
tOff	Sample injection end time (s)	Provided as input
RI	Bulk refractive index contribution in the sample	Fitted

The following values are presented in the report:

		Calculated as
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
Flow	Flow rate (µl/min)	f
kt	Mass transfer constant	$tc*f^{1/3}$
RI	Bulk refractive index contribution in the sample	RI

9.3.2 Kinetics – Bivalent Analyte

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 of often referred to as avidity.

$A+B=AB$
$AB+B=AB_2$

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 since there is no change in the amount of analyte at the surface. 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 conversion factor between RU and M is available. Similarly, a value for the overall affinity or avidity constant is not reported.

Model parameters are

		Obtained from
ka1	Association rate constant for the first site ($M^{-1}s^{-1}$)	Fitted
kd1	Dissociation rate constant for the first site (s^{-1})	Fitted
ka2	Association rate constant for the second site ($RU^{-1}s^{-1}$)	Fitted
kd2	Dissociation rate constant for the second site (s^{-1})	Fitted
Rmax	Analyte binding capacity of the surface (RU)	Fitted
Conc	Analyte concentration (M)	Provided as input
tc	Flow rate-independent component of the mass transfer constant	Fitted
f	Flow rate (µl/min)	Provided as input
tOn	Sample injection start time (s)	Provided as input
tOff	Sample injection end time (s)	Provided as input
RI	Bulk refractive index contribution in the sample	Fitted

The following values are presented in the report:

		Calculated as
ka1	Association rate constant for the first site ($M^{-1}s^{-1}$)	ka1
kd1	Dissociation rate constant for the first site (s^{-1})	kd1
ka2	Association rate constant for the second site ($RU^{-1}s^{-1}$)	ka2
kd2	Dissociation rate constant for the second site (s^{-1})	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
Flow	Flow rate (µl/min)	f
kt	Mass transfer constant	$tc*f^{1/3}$
RI	Bulk refractive index contribution in the sample	RI

9.3.3 Kinetics – Heterogeneous Analyte

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 are

		Obtained from
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
Conc1 Conc2	Concentration of the first and second analytes (M)	Provided as input
mw1 mw2	Molecular weights of the first and second analytes	Provided as input
tc1 tc2	Flow rate-independent component of the mass transfer constant for the first and second analytes	Fitted
Rmax1	Analyte binding capacity of the surface for the first analyte (RU)	Fitted
Rmax2	Analyte binding capacity of the surface for the second analyte (RU)	Fitted
rcf	Response correction factor, allowing for different refractive index contributions for the two analytes. This factor is defined as (Rmax1/Rmax2)(MW1/MW2).	Fitted
f	Flow rate (µl/min)	Provided as input
tOn	Sample injection start time (s)	Provided as input
tOff	Sample injection end time (s)	Provided as input
RI	Bulk refractive index contribution in the sample	Fitted

The following values are presented in the report:

		Calculated as
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	Analyte binding capacity of the surface for the first analyte (RU)	Rmax2*rcf*mw1/mw2

Rmax2	Analyte binding capacity of the surface for the second analyte (RU)	Rmax2
Conc1 Conc2	Analyte concentration (M)	Conc1 Conc2
tc1 tc2	Flow rate-independent component of the mass transfer constant for the first and second analytes	tc1 tc2
Flow	Flow rate (µl/min)	f
kt1 kt2	Mass transfer constants for the first and second analytes	$tc1*f^{1/3}$ $tc2*f^{1/3}$
RI	Bulk refractive index contribution in the sample	RI

9.3.4 Kinetics – Heterogeneous Ligand

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.

Heterogeneous ligand situations frequently arise in practice through heterogeneous immobilization of ligand (e.g. amine coupling of proteins, where the ligand has multiple attachment points), as well as through heterogeneity in the ligand preparation itself. In cases where the heterogeneous ligand model is found to give the best fit to the observed sensorgrams, further experimental efforts to reduce the heterogeneity are recommended where possible.

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 are

		Obtained from
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	Analyte binding capacity of the first ligand (RU)	Fitted
Rmax2	Analyte binding capacity of the second ligand (RU)	Fitted
Conc	Analyte concentration (M)	Provided as input
Tc	Flow rate-independent component of the mass transfer constant	Fitted
F	Flow rate (µl/min)	Provided as input
TOn	Sample injection start time (s)	Provided as input
tOff	Sample injection end time (s)	Provided as input
RI	Bulk refractive index contribution in the sample	Fitted

The following values are presented in the report:

		Calculated as
ka1 ka2	Association rate constant for the first and second ligands (M^{-1}s^{-1})	ka1 ka2
kd1 kd2	Dissociation rate constant for the first and second ligands (s^{-1})	kd1 kd2
KD1 KD2	Equilibrium dissociation constants (M)	kd1/ka1 kd2/ka2
Rmax1	Analyte binding capacity of the first ligand (RU)	Rmax1
Rmax2	Analyte binding capacity of the second ligand (RU)	Rmax2
Conc	Analyte concentration (M)	Conc
Tc	Flow rate-independent component of the mass transfer constant	tc
Flow	Flow rate (µl/min)	f
Kt	Mass transfer constant	tc*f1/3
RI	Bulk refractive index contribution in the sample	RI

9.3.5 Kinetics – Two State Reaction

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

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

Note that conformational changes in ligand or complex do not normally give a response in Biacore. 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 are

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

The following values are presented in the report:

		Calculated as
ka1	Association rate constant for analyte binding ($M^{-1}s^{-1}$)	ka1
kd1	Dissociation rate constant for analyte from the complex (s^{-1})	kd1
ka2	Forward rate constant for the conformational change (s^{-1})	ka2
kd2	Reverse rate constant for the conformational change (s^{-1})	kd2
KD	Overall equilibrium dissociation constant (M)	kd1/ka1* (kd2/(kd2+ka2))
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
Flow	Flow rate (μl/min)	f
kt	Mass transfer constant	tc*f1/3
RI	Bulk refractive index contribution in the sample	RI

9.3.6 Affinity – Steady State 1:1

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 a term for the bulk refractive index contribution RI, which is assumed to be the same for all samples. This term simply serves as an offset on the R_{eq}-axis.

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

Model parameters and reported results are

		Obtained from
KD	Equilibrium dissociation constant (M)	Fitted
Rmax	Analyte binding capacity of the surface (RU)	Fitted
RI	Bulk refractive index contribution in the sample	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 Section 9.1.2).

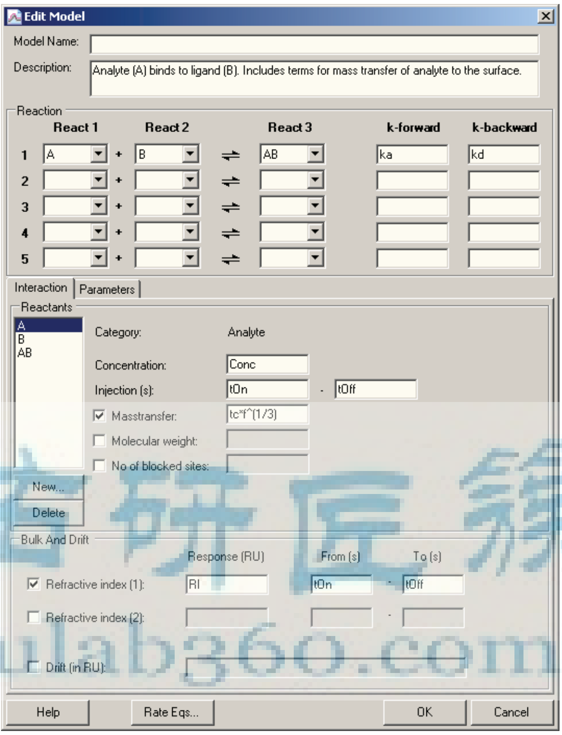
9.4 Creating and editing models

To create your own models for kinetics of 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. Interaction models are described in Section 9.4.1 and equation models in Section 9.4.2.

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.

9.4.1 Interaction models for kinetics

The reaction scheme for an interaction model supports up to 5 component reactions. Follow the steps below to define a new model or edit an existing definition.



Model Name:

Description: Analyte (A) binds to ligand (B). Includes terms for mass transfer of analyte to the surface.

Reaction

	React 1		React 2		React 3	k-forward	k-backward
1	A	+	B	⇌	AB	ka	kd
2		+		⇌			
3		+		⇌			
4		+		⇌			
5		+		⇌			

Reactants: A, B, AB

Category: Analyte

Concentration: Conc

Injection (s): tOn - tOff

☑ Masstransfer: tc*f^(1/3)

☐ Molecular weight:

☐ No of blocked sites:

Bulk And Drift

	Response (RU)	From (s)	To (s)
☑ Refractive index (1):	RI	tOn	tOff
☐ Refractive index (2):			
☐ Drift (in RU):			

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.

   Note: Numbers are used as part of the identifier, not in the conventional chemical sense of stoichiometry. Thus a complex named AB2 does not imply two molecules of B binding to one of A.

Analyte
The analyte is injected in solution at a constant concentration, and has the properties listed below. Analyte is usually denoted by the letter A.

Concentration	Injected concentration in molar units.
Injection(s)	Start and stop times for the injection.
Mass transfer	Check this box to include a mass transfer term in the fitting, and enter a parameter name or expression for the mass transfer constant.
Molecular weight	Check this box and enter a molecular weight if required. This information is used to calculate relative response contributions for heterogeneous analyte models (it is not used for conversion of weight-based to molar concentration units: this conversion is performed if necessary in the sample table).
Number of blocked sites	Check this box and enter a number if binding of one analyte molecule sterically blocks additional ligand sites. This box should normally be left unchecked.

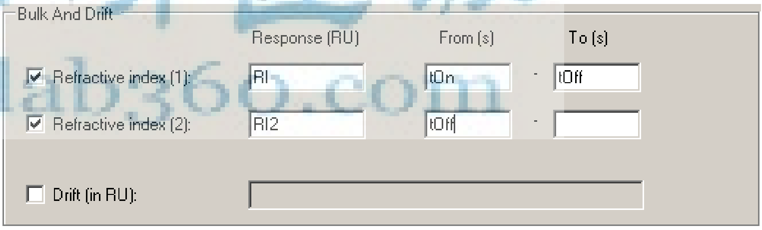
Ligand
The ligand is immobilized or captured on the surface, and has the properties listed below. Ligand is usually denoted by the letter B.

Binding capacity	Maximum analyte binding capacity of the surface in RU.
At molecular weight	This parameters is only used in heterogeneous analyte models. Check the box 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 generates response and has the properties listed below.

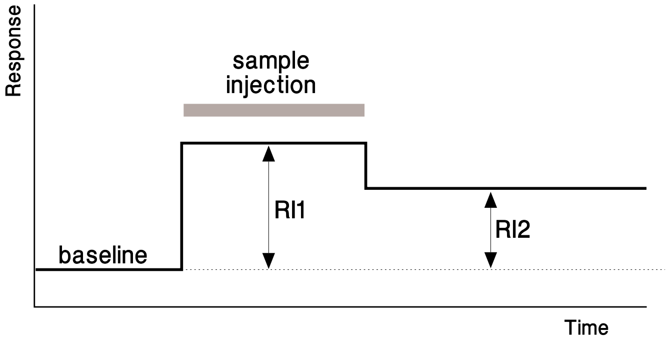
Generates response	Uncheck this box for complexes that form in solution and that do not contribute to the response.
Molecular weight	check this box and specify a parameter for complexes that form in solution and then bind to the surface. Do not check this box if **Generates response** is also checked.

In the **Bulk and Drift** panel, enter details for bulk refractive index contribution. Normally, there will be one bulk refractive index term applicable from the start to the end of the injection. A second term can be used if necessary: for example, enter a second refractive index term with the injection end in the **From** box and the **To** box left blank to accommodate a permanent shift in baseline as a result of the sample injection:



Bulk And Drift

	Response (RU)	From (s)		To (s)
☑ Refractive index (1):	RI	tOn	-	tOff
☑ Refractive index (2):	RI2	tOff	-	
☐ Drift (in RU):				



Check the **Drift** box and enter an expression describing the drift (most commonly a linear function of time) to account for baseline drift.

2. 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.

3. 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 dialog:

Name: ka2
Default Type: Fit global
Initial Value
● Value: 1e5
○ Attach to:
☐ Allow negative value
Description:
Second association rate constant

Choose a default type for the parameter (**Fit global**, **Fit local** or **Constant**.

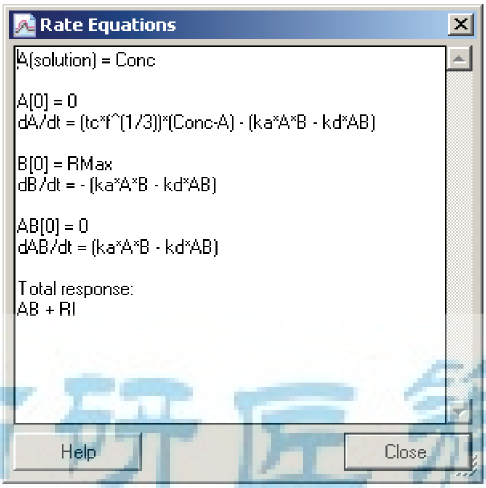
For the **Initial value**, enter a numerical value or select a value expression from the pull-down list. The expression represent functions evaluated within the current data set (e.g. **Ymax** is the maximum y-value in the data set). Alternatively, choose **Attach to** and select a parameter from the 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 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 enter suggested definitions for all undefined parameters. This shortcut cannot be used if you have entered expressions.

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.

4. Click **Rate Equations** to display the equations generated by the software.

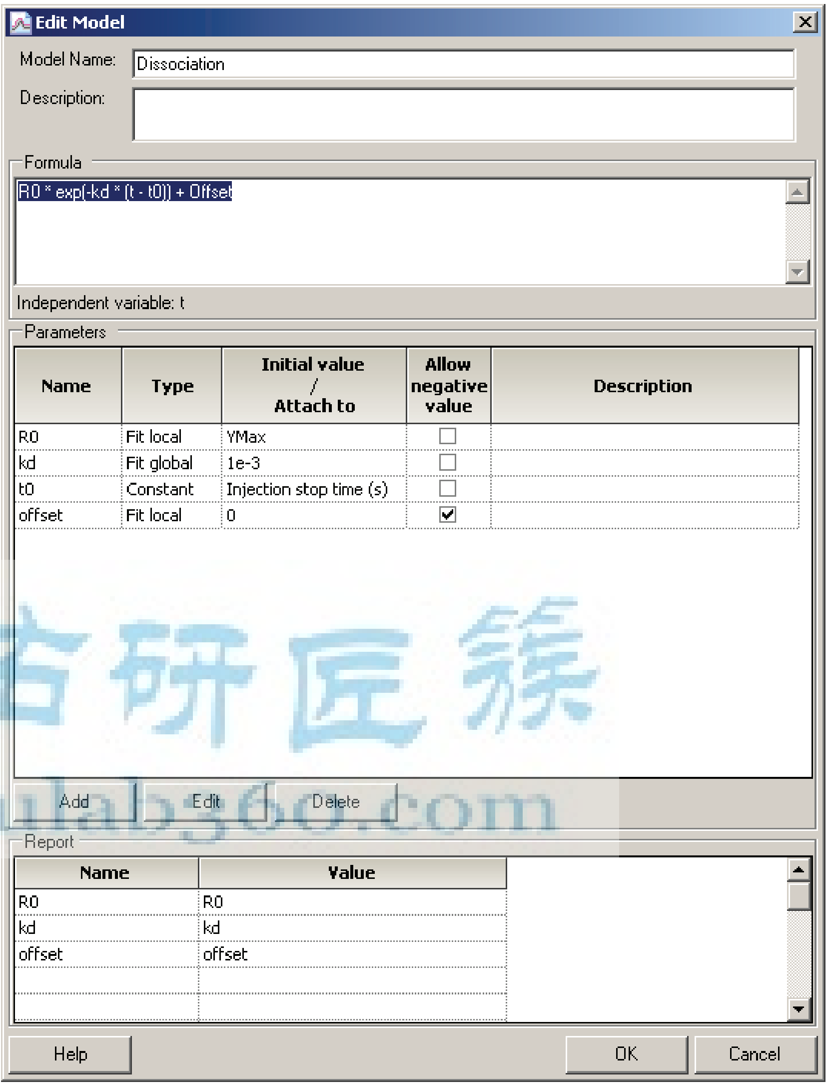


```
A(solution) = Conc

A[0] = 0
dA/dt = (tc*f^(1/3))*(Conc-A) - (ka*A*B - kd*AB)

B[0] = RMax
dB/dt = - (ka*A*B - kd*AB)

AB[0] = 0
dAB/dt = (ka*A*B - kd*AB)

Total response:
AB + RI
```

You can select the equations in the display and click **Copy** to copy the equations to the Windows clipboard. Use this function and paste the equations in to e.g. Wordpad to print a copy of the rate equations.

9.4.2 Equation models for kinetics

Models for steady state affinity evaluation are entered as an expression defining response as a function of time t. To create an equation model, choose **New** in the kinetics models dialog, and create a new model without using the currently selected model as a template.



Model Name: Dissociation

Description:

Formula

R0 * exp(-kd * (t - t0)) + Offset

Independent variable: t

Parameters

Name	Type	Initial value / Attach to	Allow negative value	Description
R0	Fit local	YMax	☐	
kd	Fit global	1e-3	☐	
t0	Constant	Injection stop time (s)	☐	
offset	Fit local	0	☑	

Report

Name	Value
R0	R0
kd	kd
offset	offset

Parameters and report parameters are defined in the same way as for kinetic models.

9.4.3 Models for steady state affinity

Models for steady state affinity evaluation are entered as an expression defining R_{eq} as a function of concentration Conc.

Model Name: Two site affinity

Description: Steady state affinity with two ligand sites

Formula

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

Independent variable: Conc

Parameters

Name	Type	Initial value	Allow negative value	Description
KD1	Fit global	XMax/2	☐	
Rmax1	Fit local	YMax/2	☐	
KD2	Fit global	XMax/2	☐	
Rmax2	Fit local	YMax/2	☐	

Report

Name	Value
KD1	KD1
Rmax1	Rmax1
KD2	KD2
Rmax2	Rmax2

Parameters and report parameters are defined in the same way as for kinetic models.

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 (typically about 5 concentrations in duplicate), complex models tend to give unstable fitting behaviour.

10. Thermodynamic analysis

Biacore T100 supports automated measurement of kinetics or affinity at a series of temperatures using the **Thermodynamics** wizard (Section 4.5.4). In addition to displaying the variation of kinetic and affinity constants with temperature, the evaluation software extracts standard thermodynamic parameters from the data.

10.1 Background

10.1.1 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

ΔG°	is the standard free energy change
R	is the universal gas constant
T	is the absolute temperature (K)
K_D	is the 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

ΔH°	is the standard enthalpy change
ΔS°	is the 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 $\Delta S^\circ/R$.

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

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

where ΔC^o_p is the heat capacity change under standard conditions

and T_0 is the reference temperature (25°C = 298.15 K for standard conditions)

A value for the standard heat capacity change ΔC^o_p can thus be obtained in addition to ΔH° and ΔS° from non-linear fitting of the data to this extended equation.

10.1.2 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

$K^{\ddagger}$	is the equilibrium constant for formation of the transition state for the forward or back reaction
k	is the kinetic rate constant for the interaction in the corresponding direction (k_a or k_d)
$\hbar$	is Planck's constant
k_B	is Boltzmann's constant

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

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

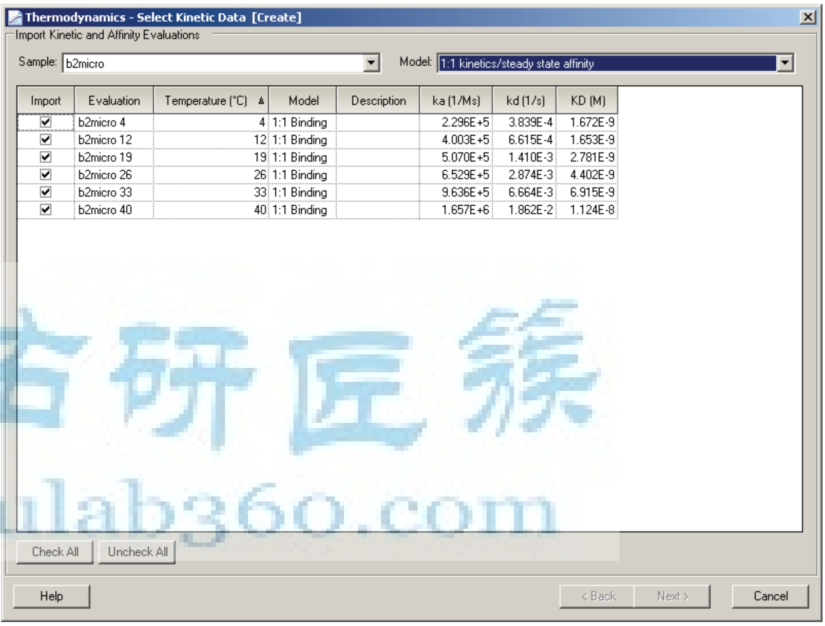
so that the thermodynamic transition state constants for the forward and backward reactions can be obtained from plots of $\ln(k_a/T)$ and $\ln(k_d/T)$ respectively against 1/T.

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 ΔC^o_p can only be applied to equilibrium thermodynamic analysis.

10.2 Performing thermodynamic analysis

Before thermodynamic analysis can be performed for a set of data, the kinetics and/or affinity must be evaluated at each temperature. Create a separate **Kinetics/Affinity** evaluation item at each temperature used in the run, using the same fitting model for each item (see Section 9.1.2).

When all required kinetic and affinity evaluation items have been created, click **Thermodynamics** on the toolbar.



Sample: b2micro

Model: 1:1 kinetics/steady state affinity

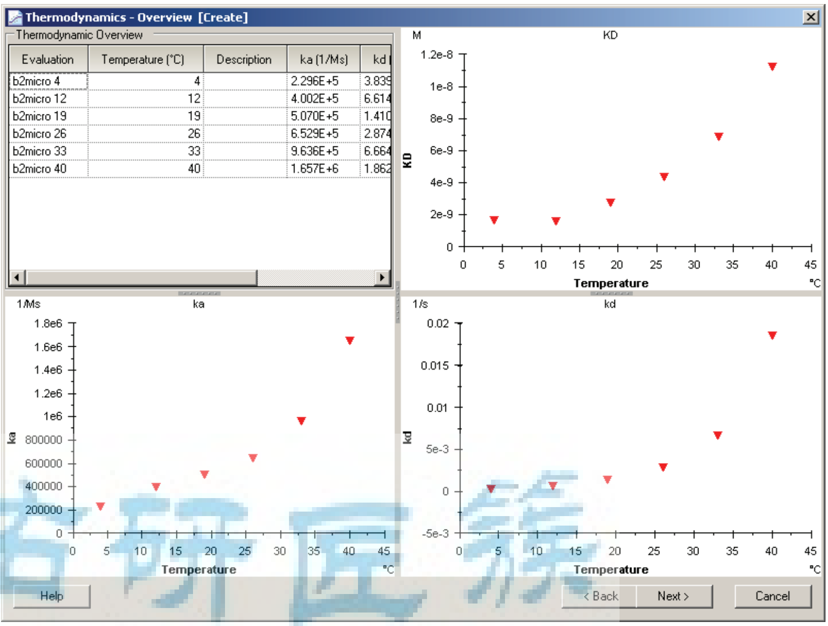
Import	Evaluation	Temperature (°C)	Model	Description	ka (1/Ms)	kd (1/s)	KD (M)
☑	b2micro 4	4	1:1 Binding		2.296E+5	3.839E-4	1.672E-9
☑	b2micro 12	12	1:1 Binding		4.003E+5	6.615E-4	1.653E-9
☑	b2micro 19	19	1:1 Binding		5.070E+5	1.410E-3	2.781E-9
☑	b2micro 26	26	1:1 Binding		6.529E+5	2.874E-3	4.402E-9
☑	b2micro 33	33	1:1 Binding		9.636E+5	6.664E-3	6.915E-9
☑	b2micro 40	40	1:1 Binding		1.657E+6	1.862E-2	1.124E-8

Choose the sample and the fitting model in the pull-down lists. You may only choose one sample, and you should only choose one fitting model. Options for the model are **1:1 kinetics/steady state affinity** (recommended) or **All**. If you choose **All** it is possible to combine data from different fitting models in the same evaluation: however, values for thermodynamic constants are in all likelihood meaningless if the data is obtained from a mixture of different models.

Check the rows for data that you want to use in the thermodynamic evaluation. Use the **Check All** and **Uncheck All** as quick options to select and deselect the whole list.

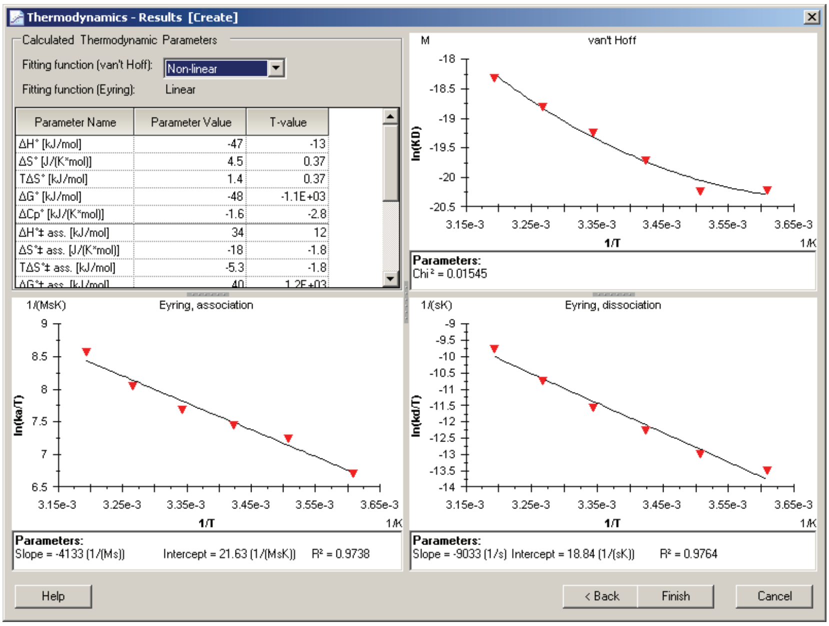
Note: If you use data from fitting models that include multiple rate or affinity constants, be sure to select the correct rows so that equivalent constants are included from each fit. In some cases it may be necessary to examine the kinetic or affinity evaluation items to determine which constants belong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

Click **Next>** when you have selected the data to be included. The results are displayed first as plots of affinity and rate constants against temperature.



Evaluation	Temperature (°C)	Description	ka (1/Ms)	kd
b2micro 4	4		2.296E+5	3.83
b2micro 12	12		4.002E+5	6.61
b2micro 19	19		5.070E+5	1.41
b2micro 26	26		6.529E+5	2.87
b2micro 33	33		9.636E+5	6.66
b2micro 40	40		1.657E+6	1.86

Click **Next>** to display the van't Hoff and Eyring plots together with a table of thermodynamic constants for the equilibrium and transition state formation. In any of the plots, right-click on a point to exclude the point from the line fitting.



Fitting function (van't Hoff): Non-linear

Fitting function (Eyring): Linear

Parameter Name	Parameter Value	T-value
ΔH° [kJ/mol]	-47	-13
ΔS° [J/(K*mol)]	4.5	0.37
TΔS° [kJ/mol]	1.4	0.37
ΔG° [kJ/mol]	-48	-1.1E+03
ΔCp° [kJ/(K*mol)]	-1.6	-2.8
ΔH°‡ ass. [kJ/mol]	34	12
ΔS°‡ ass. [J/(K*mol)]	-18	-1.8
TΔS°‡ ass. [kJ/mol]	-5.3	-1.8
ΔG°‡ ass. [kJ/mol]	40	1.2E+03

Parameters:
Chi² = 0.01545

Parameters:
Slope = -4133 (1/(Ms)) Intercept = 21.63 (1/(MsK)) R² = 0.9738

Parameters:
Slope = -9033 (1/s) Intercept = 18.84 (1/(sK)) R² = 0.9764

Choose whether to use a linear or non-linear fitting function for the van't Hoff plot (see Section 10.1.1) 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.

In any of the plots, right click on a point to exclude it from the evaluation.

Notes: 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 Section 10.1.2).

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).

Click on **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 role of mass transport limitations in the observed interaction) 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.

11. Affinity in solution

Determination of affinity in solution provides an alternative to steady state affinity measurements (see Chapter 9) 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 to determine the free concentration of one interactant in equilibrium mixtures containing known total interactant concentrations.

11.1 Conventions and background

11.1.1 Experimental setup

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

$$\mathrm{A + B = AB}$$

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 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.

11.1.2 Evaluation principles

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

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

or

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

Rearranging gives

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

or

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

Solving for AB:

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

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

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

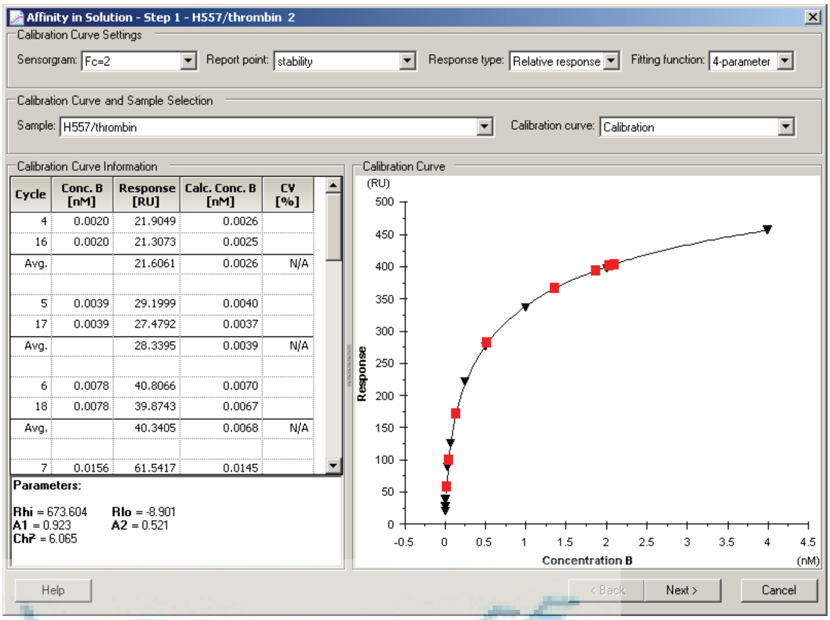
This equation can be fitted to a plot of B_{free} against A_{tot} to calculate a value for K_D.

11.2 Requirements for affinity in solution

Affinity in solution experiments are run using a method. The method must be correctly constructed as described in Section 5.10.4: if necessary, the keyword table can be edited so that the conditions are met in full (see Section 6.6). Note however that the command name cannot be edited in the keyword table. Refer to Chapter 5 for details of how to construct methods in Method Builder. Determination of affinity in solution is not supported by a wizard.

11.3 Evaluation of affinity in solution

To evaluate affinity in solution measurements, open the result file and click **Affinity in Solution** on the toolbar. The first step displays the calibration curve for measurement of free B:



Sensorgram: Fc=2 Report point: stability Response type: Relative response Fitting function: 4-parameter

Sample: H557/thrombin Calibration curve: Calibration

Cycle	Conc. B [nM]	Response [RU]	Calc. Conc. B [nM]	CV [%]
4	0.0020	21.9049	0.0026	
16	0.0020	21.3073	0.0025	
Avg.		21.6061	0.0026	N/A
5	0.0039	29.1999	0.0040	
17	0.0039	27.4792	0.0037	
Avg.		28.3395	0.0039	N/A
6	0.0078	40.8066	0.0070	
18	0.0078	39.8743	0.0067	
Avg.		40.3405	0.0068	N/A
7	0.0156	61.5417	0.0145	

Parameters:

Rhi = 673.604 **Rlo** = -8.901
A1 = 0.923 **A2** = 0.521
Chi² = 6.065

Choose the sensorgram, report point, response type and fitting function from the pull-down lists at the top of the dialog. See Section 8.2.1 for more details of these choices.

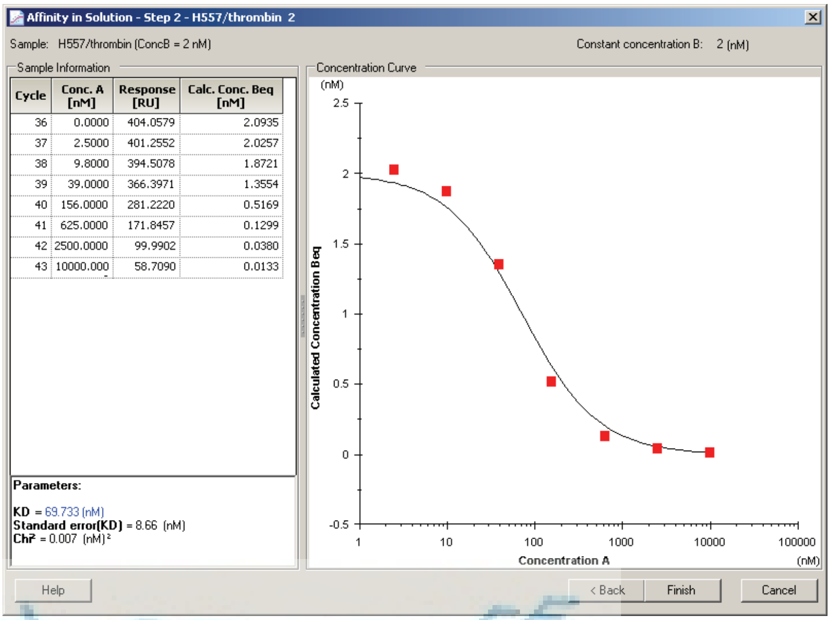
If you have run multiple sample series in the experiment, choose the sample to evaluate in the **Sample** list. A sample series is defined as all cycles with the same sample name in the assay step(s) with purpose **Sample**.

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.

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.

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



Affinity in Solution - Step 2 - H557/thrombin 2

Sample: H557/thrombin (ConcB = 2 nM)

Constant concentration B: 2 (nM)

Sample Information

Cycle	Conc. A [nM]	Response [RU]	Calc. Conc. Beq [nM]
36	0.0000	404.0579	2.0935
37	2.5000	401.2552	2.0257
38	9.8000	394.5078	1.8721
39	39.0000	366.3971	1.3554
40	156.0000	281.2220	0.5169
41	625.0000	171.8457	0.1299
42	2500.0000	99.9902	0.0380
43	10000.000	58.7090	0.0133

Parameters:

KD = 69.733 (nM)
Standard error(KD) = 8.66 (nM)
Chi² = 0.007 (nM)²

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** (not applicable) in the column for **Calc.Conc.Beq**.

The plot panel shows the sample measurements with a line fitted according to the equation for 1:1 affinity (see Section 11.1.2). Right-click on a point to exclude it from the fitting.

Notes: The plot of free B against total A is presented by default with a logarithmic scale on the x-axis. In this form, the equilibrium constant KD is given by the concentration of A at the inflection point in the fitted curve.

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.

The intercept of the fitted curve on the y-axis represents a fitted value for the parameter **ConcB**. This value should be the same as or close to the value entered for the variable **ConcB** in the method.

The calculated K_D value is shown in the panel below the table.

A. Data import and export

This appendix describes the functions and data format for data import and export.

A.1 Exporting data

A.1.1 Export functions

Data can be exported from both the Control and Evaluation Software in Microsoft Excel or Extended Markup Language (XML) format. The report point table can be exported to a tab-separated text file. Rack positions may also be exported from the **Rack Positions** dialog box.

Export to Excel

To export data to an Excel file, choose **File:Export:Results 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. The audit trail is also exported to a separate worksheet if it is present when the GxP module is installed (see the separate Biacore T100 GxP Handbook).

Export from the Evaluation software 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. The audit trail is also exported to a separate worksheet if it is present when the GxP module is installed (see the separate Biacore T100 GxP Handbook).

Export to XML

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

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

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**.

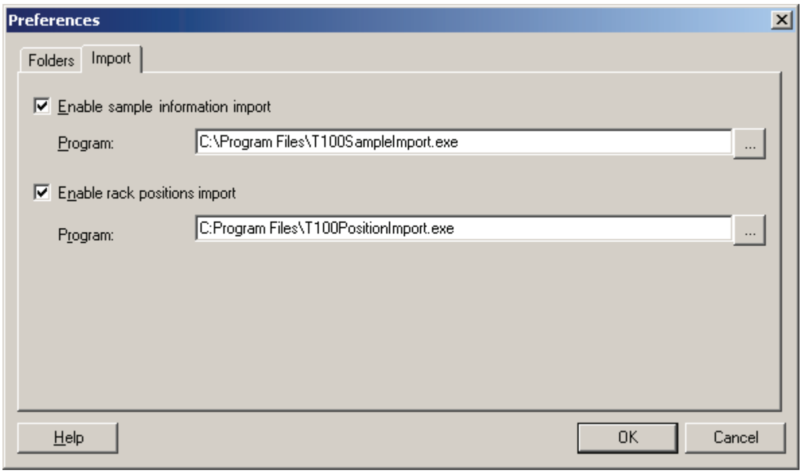
Note: If you open an exported report point table in Microsoft Excel, make sure that the format for the **Fc** column is set to **Text** in the Excel import file wizard. The default setting of **General for** text file import may interpret the flow cell identification for reference-subtracted data as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

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 (Section 4.2.5). 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

A.2.1 Control Software

Sample table import
The Control Software supports data import to sample tables in assay wizard templates and in the **Setup Run** step of methods. In order to use the import function, the option must be activated in **Tools:Preferences** and an import program must be specifed.



☑ Enable sample information import

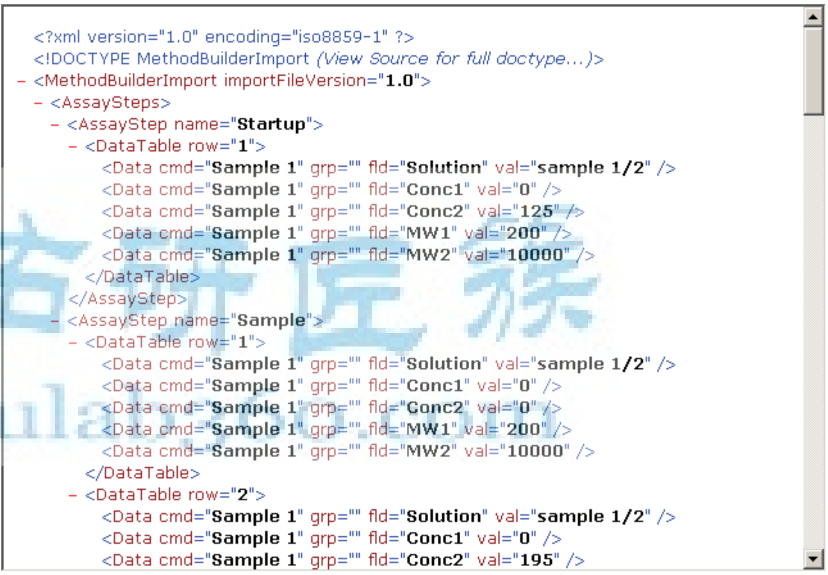
Program: C:\Program Files\T100SampleImport.exe

☑ Enable rack positions import

Program: C:Program Files\T100PositionImport.exe

When the import function is requested from a dialog box, the contents of the table are first exported in Extended 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.



```
  <?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" />
```

Part of an XML import file from a method for heterogeneous analyte kinetics displayed in an XML-compatible editor.

Rack positions import

The **Menu:Import Positions** function in the **Rack Positions** dialog (Section 4.2.5) imports rack position data from an external file such as one from a laboratory robot used to prepare sample microplates. 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 following specification:

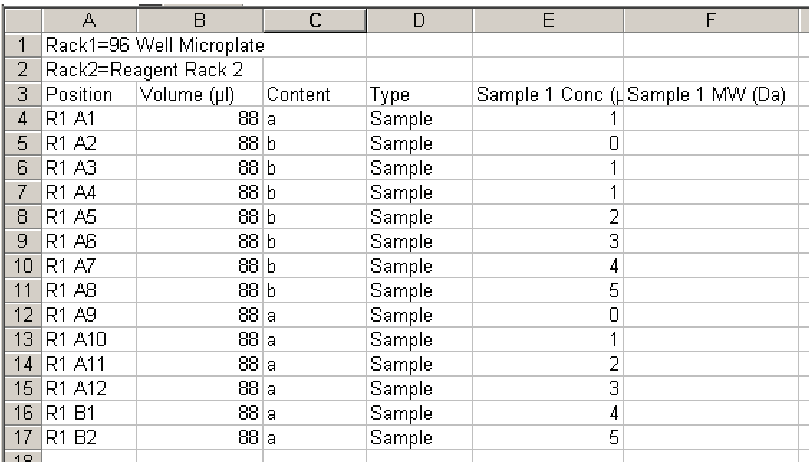
- Two lines in the file specify the microplate and reagent rack settings, in the format
  `Rack1=<microplate specification>`
  `Rack2=<reagent rack specification>`
  Specifications are not case-sensitive, but microplate and reagent rack specifications must be given otherwise exactly as they appear in 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 nor 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.

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.

Details of the required import file format can be investigated further by examining a file created with the **Menu:Export Positions** command.



	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 (	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	

Example of an exported file for rack positions, opened in Microsoft Excel.

A.2.2 Evaluation Software

The Evaluation Software supports import of model definitions for kinetics and affinity evaluation. Model files for import should be obtained from Biacore or created by exporting models from another installation.

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