HANDBOOK

# Biacore®X100


BIACORE

# Biacore® X100 Handbook

Edition May 2007

Biacore® X100 Handbook
Edition May 2007 (Version AA).



Biacore AB reserves the right to make changes to the design and specification of Biacore X100 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

# Safety

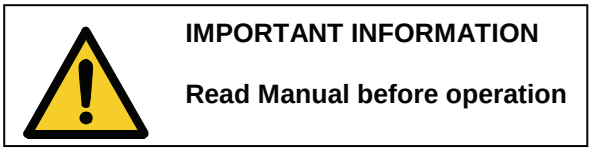
## Safety information

Biacore X100 Instrument contains mains voltage and handles liquids that may be hazardous. Before operating or maintaining the system, **you must read this handbook and be aware of the hazards described below.** Follow the instructions provided to avoid personal injuries or damage to the equipment. Do not use the equipment in any other way than described in this manual.

### *Rating information*

Instrument ratings are printed on the mains input panel at the rear of the instrument.

### *Safety labels*




This safety label is attached to the rear panel of the instrument, above the communication ports.

### *Safety instructions*

*WARNING!* *QUALIFIED OPERATION*
**Biacore X100 should only be operated by properly qualified personnel. Read this manual before operating the instrument.**

*WARNING!* *HAZARDOUS VOLTAGES*
**Biacore X100 Instrument contains mains voltage of up to 265 V ac. Disconnect mains cord before replacing fuses. Do not remove instrument covers.**

*WARNING!* *GENERAL PROCEDURES*
**Always wear appropriate protective clothing during operation and maintenance of Biacore X100.**
**Use required safety equipment when handling hazardous substances.**

*WARNING!* *FLAMMABLE SUBSTANCES*
**Liquids marked as flammable must not be used as running buffer. Any buffer or reagent containing flammable substances must be placed in properly capped vials in the sample rack.**

*WARNING!* *SHARP NEEDLE*
**The injection needle is sharp. Take care when working in the sample compartment.**

*WARNING!* *MOVING NEEDLE*
**Do not put your hands in the sample compartment while the Rack Locked lamp is lit.**

*WARNING!* *MOVING PUMP*
**Do not touch the pumps while they are moving.**

*WARNING!* *HEAVY OBJECT*
**Biacore X100 weighs 47 kg. Do not try to lift the instrument on your own.**

### *Disposal procedures*

Follow applicable national and/or local regulations for the disposal of chemicals and other materials.

This symbol indicates that electrical and electronic equipment must not be disposed of as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of your equipment.

Biacore X100 contains a lithium backup battery, which must not be disposed of in fire.

### Emergency stop routine

Choose **Run:Stop Run** from the menu bar to stop a run under controlled conditions before it is complete. This will stop both the run and the data collection at the end of the current cycle. A dialog is displayed while the current cycle is finished.

*In an emergency situation*

1. Press **Ctrl-Break** (**Ctrl-Pause**) on the keyboard to stop the run and the data collection immediately in an emergency situation.

> *Caution!*
> Do not use **Ctrl-Break** unless there is a risk of injury, damage or loss of valuable material. All operations including buffer flow and data collection are stopped immediately.

2. In the dialog box that appears, click **Yes** if you want to wash the system with running buffer.
   You should do this if possible. The wash operation takes about 3 minutes.

> *Caution!*
> Do not leave the system in an emergency stop condition. Always follow the restart procedure if possible, to restore the instrument into normal condition.

### Restart procedure

1. Turn on mains power and check that the instrument starts normally (see Section 3.1).

2. If you need to clean the liquid handling system, eject the sensor chip and insert a maintenance chip. See Chapter 7 for further instructions.

## Conformance with standards

### *Europe*

Biacore X100 meets the requirements of the following directives, through the referenced harmonized standards:

| European directive | Harmonized standard |
|---|---|
| 73/23/EEC, Low voltage devices | IEC/EN 61 010-1 (2001), IEC 61 010-2-081 (2001), EN 61 010-2-081 (2002), Safety requirements for electrical equipment for measurement, control and laboratory use. |
| 89/336/EEC, Electromagnetic compatibility | EN 61 326 (1997) and amendments A1 (1998), A2 (2001), and A3 (2003), EMC requirements for Class B equipment. |
| 2002/96/EC Waste Electrical and Electronic Equipment (WEEE) | - |

### *North America*

Biacore X100 meets the following safety standards:

- CAN/CSA-C.22.2 No 61010.1, 2nd edition (2004), CAN/CSA-C.22.2 No 61010-2-081:04 and UL 61010-1, 2nd edition (2004 including revision 2005), Safety requirements for electrical equipment for measurement, control and laboratory use.

### *External equipment*

Any external equipment (e.g. computer) connected to Biacore X100 instrument must comply with IEC/UL 60950-1 and other applicable IEC/EN standards.

### *CE marking*

The product is CE-marked 2007 according to 93/68/EEC.

# 1. Introduction and system overview

## 1.1 Biacore X100 system

Biacore® X100 system consists of an instrument controlled from a PC running Biacore X100 Control Software. Results are evaluated using Biacore X100 Evaluation Software. The Control Software is wizard based for ease of use. Integrated support is provided in the software with methodology recommendations, tips and troubleshooting. The optional Biacore X100 Plus Package offers extra functionality and flexibility.


**Figure 1-1**. *The Biacore X100 instrument.*

### *1.1.1 Monitoring interactions*

Biacore X100 system monitors molecular interactions in real time using label free detection based on the phenomenon of surface plasmon resonance (SPR). One of the interacting molecules is immobilized on the surface of a sensor chip, while the other is injected in solution and flows over the sensor surface.

As molecules from the injected sample bind to the immobilized molecules, this results in an alteration in refractive index at the sensor surface that is proportional to the change in mass concentration. These changes are detected in real time and data is presented in a sensorgram (SPR response plotted against time), see Figure 1-2. The sensorgram displays binding curves over the entire course of an interaction and

reveals association and dissociation rates of the interaction. Binding response is measured in resonance units (RU). Binding responses at specific times during the interaction are called report points.

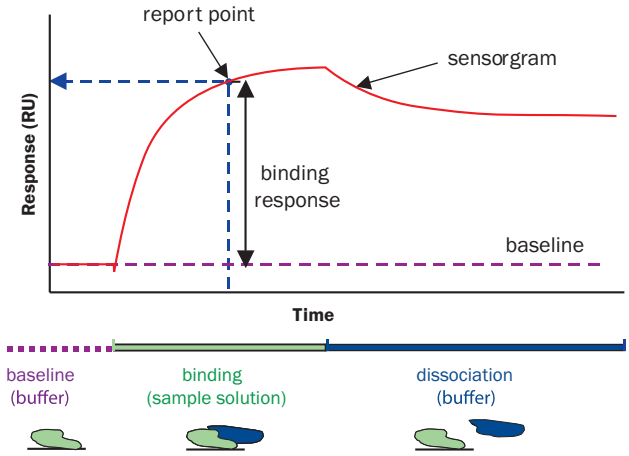



**Figure 1-2.** *Sensorgram and interaction.*

### 1.1.2 General assay principles

Setting up an assay with Biacore X100 involves preparing the sensor surface by attachment of ligand and establishment of suitable conditions for regeneration if necessary. All of these steps are performed with the sensor chip in place in the instrument. Sensor chips can normally be re-used for several runs if required.

To analyze samples with Biacore X100, sample solution is injected over the sensor surface using automated sample handling facilities. All steps in surface preparation, analysis and regeneration are monitored in a sensorgram, which records changes in the molecular concentration at the sensor surface with time. Binding is monitored in real time as the sample passes over the surface.

At the end of the sample injection, sample is replaced by a flow of running buffer and bound sample can dissociate from the surface. If the dissociation rate is rapid, sample dissociates completely within a short time and the surface can be used directly for the next analysis. For slower dissociation rates, the surface can be washed with an injection of regeneration solution designed to remove bound sample (Figure 1-4).

## 1.2 Biacore terminology

Biacore systems monitor the interaction between two molecules, of which one is attached to the sensor surface and the other is free in solution. The following terms are used in the context of Biacore assays:

- The interaction partner attached to the surface is called the ***ligand*** (Figure 1-3). (The term "ligand" is applied here in analogy with terminology used in affinity chromatography contexts, and does not imply that the surface-attached molecule is a ligand for a cellular receptor.)

- Ligand may be attached to the surface either by covalent ***immobilization*** using chemical coupling reagents or by capturing through high affinity binding to an immobilized ***capturing molecule***. Adsorption to a hydrophobic surface covered with a lipid layer can also be used to attach membrane-associated molecules.

- The ***analyte*** is the interaction partner that is passed in solution over the immobilized ligand (Figure 1-3).

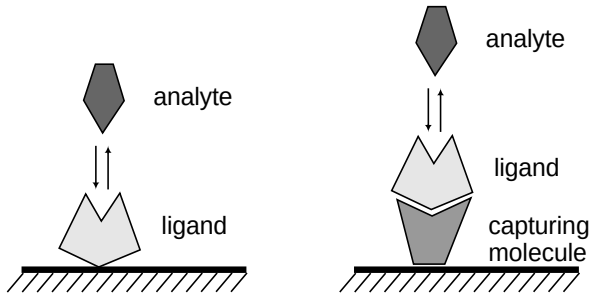



**Figure 1-3.** *The ligand is the interaction partner that is attached to the sensor surface. Ligand may be immobilized directly on the surface (left) or attached through binding to an immobilized capturing molecule (right). The analyte is free in solution and binds to the immobilized ligand.*

- Analysis is performed by injecting sample over the surface in a carefully controlled fashion. The sample is carried in a continuous flow of buffer, termed ***running buffer***.

- ***Regeneration*** is the process of removing bound analyte from the surface after an analysis cycle without damaging the ligand, in preparation for a new cycle. For ligand that is captured rather than immobilized, regeneration usually removes the ligand and leaves the capturing molecule intact.

- Response is measured in ***resonance units*** (RU). The response is directly proportional to the concentration of biomolecules on the surface.

- A ***sensorgram*** is a plot of response against time, showing the progress of the interaction (Figure 1-4). This curve is displayed directly on the computer screen during the course of an analysis.
- A ***report point*** records the response level and sensorgram slope at a specific time averaged over a short time window. The response may be absolute (above a fixed zero level determined by the detector) or relative to the response at another specified report point (Figure 1-4).

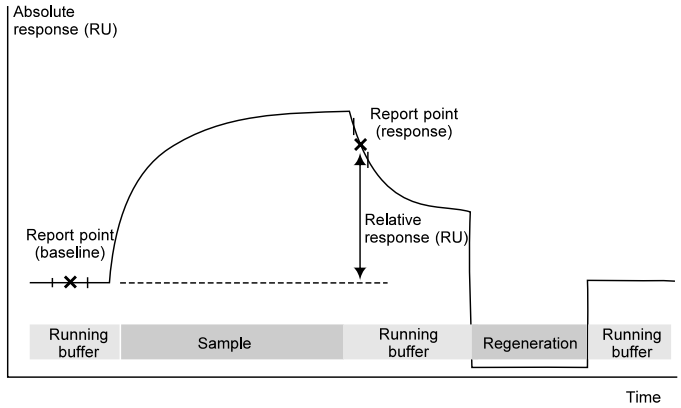



**Figure 1-4.** *Schematic illustration of a sensorgram. The bars below the sensorgram curve indicate the solutions that pass over the sensor surface.*

## 1.3 Learning to use Biacore X100

If you are a new user it is recommended to follow the link **Learning to use Biacore X100** from the start page in the Support Navigator. From there, you can get to know the instrument by:

- viewing a short movie about Biacore X100
- taking a free course on the Biacore web site
- performing a guided assay in the Getting Started Tutorial using a Getting Started Kit (kit ordered separately)

## 1.4 Biacore X100 instrument overview

Figure 1-5 illustrates the main components of Biacore X100 instrument.

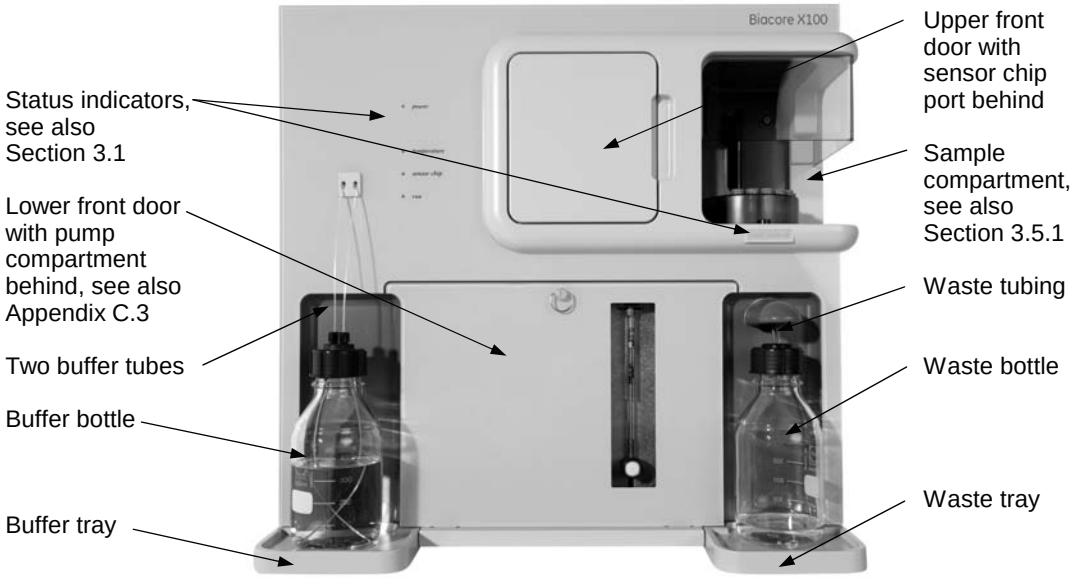



**Figure 1-5.** *Biacore X100 instrument components.*

## 1.5 Biacore X100 software overview

Biacore X100 software is designed to make the system easy and efficient for both new and experienced users. All information is conveniently collected and stored in the Biacore X100 database which can be stored locally or via a network depending on your setup.

### *1.5.1 Biacore X100 Software*

Biacore X100 Control Software controls the instrument and supports assay development and various assays. Biacore X100 Evaluation Software is used to analyze the data from assay runs. Support for using the software as well as methodology advice for setting up your experiments and evaluating the data is integrated with the software. For more information on assay setup, see Chapter 4. For more information on evaluation, see Chapter 5.

The optional Biacore X100 Plus Package is a combined hardware and software package that enables variable assay temperature control, concentration analysis and custom assay formats including single-cycle kinetics. For more information on Biacore X100 Plus Package, see Chapter 6.

### 1.5.2 Getting support

Support for using the software and methodology advice for setting up and evaluating your experiments is integrated with the software. The support includes direct links to more in-depth information from Biacore's extensive knowledge base (these links require access to the Internet). Internet access is recommended on the computer controlling the instrument if this is possible. The knowledge base can also be accessed from the Biacore web site at www.biacore.com (the product key for your system is required as authorization for web-based access).

# 2. Biacore X100 basics

This chapter provides an overview of the type of assays and assay formats that can be used with Biacore X100. More in-depth information is accessible via the Support Navigator in Biacore X100 Control and Evaluation Software and also at www.biacore.com.

## 2.1 Available assays with Biacore X100

With Biacore X100 you can perform kinetics/affinity analyses, binding analyses and (with the optional Biacore X100 Plus Package) concentration analyses.

### *2.1.1 Kinetic/Affinity analysis*

Use this assay to measure the kinetics (association and dissociation rates) and/or affinity (binding strength) of a ligand-analyte interaction. Affinity can be obtained from measurement of steady-state binding levels or as the ratio of kinetic rate constants.

The standard assay is a multi-cycle assay where you run several cycles over a range of analyte concentrations. Each sample concentration is run in a separate cycle. Analyte is removed by dissociation or regeneration at the end of each cycle.

If you have the Biacore X100 Plus Package installed, you may run single-cycle assays where the analyte is injected with increasing concentrations in a single cycle. The surface is not regenerated between injections. See more information in Section 6.3 and in the Support Navigator in the Biacore X100 Control Software.

Evaluation of kinetics/affinity analysis in the Evaluation Software calculates values for rate and affinity constants from the sensorgram data.

### *2.1.2 Binding analysis*

Use this assay to investigate analyte binding characteristics to ligand, such as binding specificity, comparative ranking studies etc. The results are typically based on report point values, not complete association and dissociation phases and are evaluated via plots and bar charts in the Evaluation Software.

Binding analysis experiments with several sample injections in the same cycle can be used to investigate the formation of multimolecular complexes.

### 2.1.3 Concentration analysis

This assay is only available if you have the Biacore X100 Plus Package installed. Use this assay to measure analyte concentration in samples, using known samples to create a calibration curve. The principles of concentration measurement with Biacore are discussed in detail in the Biacore Concentration Analysis Handbook.

A concentration analysis must include at least one calibration curve.

## 2.2 Interacting molecules in the assay

All interaction studies in Biacore X100 work with one molecule attached to the sensor surface and the other molecule in the injected sample solution. Practically any biological molecule can be studied, ranging from proteins and peptides to nucleic acids, carbohydrates and lipids, and including larger structures such as cells and viruses.

### 2.2.1 Attachment methods

There are two methods to attach a molecule to the sensor surface: capture and direct immobilization. The choice of method is discussed in some more detail in Section 2.2.3.

*Capture*

A capturing molecule is immobilized covalently on the surface in order to attach ligand by high affinity binding. The capturing molecule remains on the surface between analysis cycles. Fresh ligand is captured for each cycle.

*Direct immobilization*

The ligand is the interacting component that is covalently attached to the surface. Immobilized ligand remains on the surface between analysis cycles.

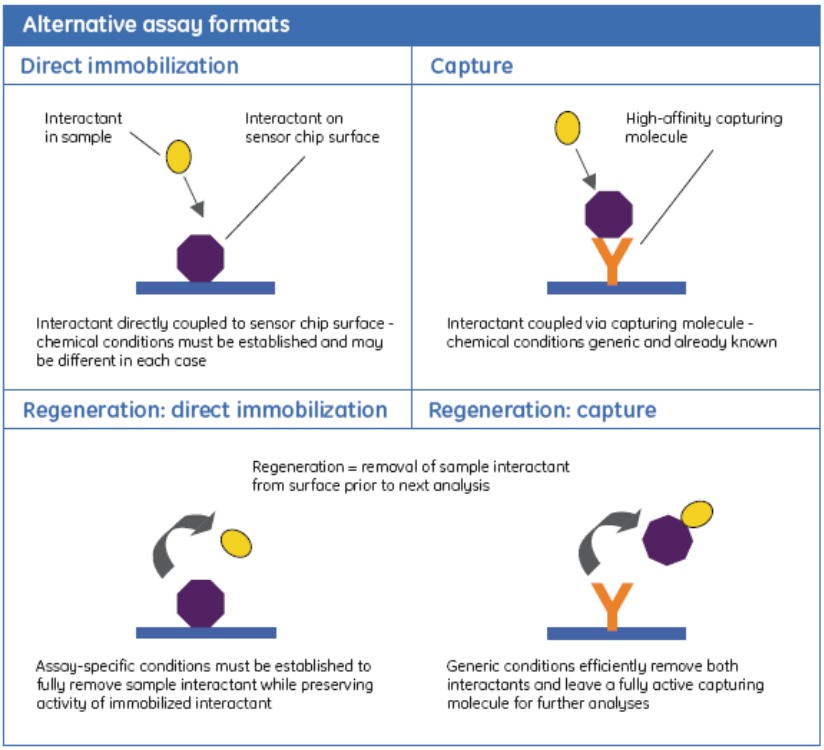



**Figure 2-1.** *Alternative assay formats.*

### 2.2.2 Overview of sensor chips

There are several sensor chip types that might be used for an assay. The selection depends on the properties of the interactants and the attachment method for the ligand.

Sensor Chip CM5 is the most widely applicable general-purpose sensor chip, and carries a surface matrix of carboxymethyl dextran to which the ligand can be covalently attached. Other sensor chips in the CM-family use the same attachment principles but differ in the detailed properties of the surface matrix.

For lipids and membrane preparations Sensor Chip HPA or Sensor Chip L1 may be a good choice.

For tagged ligands (e.g. GST, his, biotin) there are several sensor chips and reagent kits available for capturing the ligand, depending on the nature of the tag.

You can find more information in the Support Navigator in the Biacore X100 Control Software or on www.biacore.com.

### 2.2.3 Tips on how to select which molecule to attach

Many factors can influence the decision on which interactant to attach to the surface and which attachment approach to use. Some or all of the following considerations may be relevant in any individual situation:

- With covalent immobilization methods, ligand is attached once for the whole assay, so ligand consumption is kept to a minimum. On the other hand, assay development work will probably be necessary to establish suitable conditions for both immobilization and ligand regeneration, costing time and material.

- With captured ligand, using capturing kits or well-characterized reagents, conditions for immobilization and regeneration of the capturing molecule are usually known so assay development requirements are reduced. On the other hand, fresh ligand needs to be captured for each analysis cycle, taking more time for the assay and requiring larger amounts of ligand.

- Tagged recombinant proteins can often be captured using interaction with the tag. Kits and sensor chips are available from Biacore for capture with a number of common tags.

- If you want to hold one interactant constant and vary the other, it is usually most convenient to attach the constant interactant to the surface. A capturing approach is valuable if the constant interactant cannot be attached to the surface or if you want to vary both interactants, since the captured ligand can be changed between cycles (only in Custom Assay Wizard).

- Large molecules such as proteins are often easier to immobilize than small molecules, and also run less risk of the attachment interfering with the interaction being studied. On the other hand, small molecules give inherently lower sample responses that may be more difficult to measure with confidence.

- Capture is often unsuitable for attaching small molecules since the approach requires two separate binding sites on the molecule, one for the capturing interaction and one for the interaction being studied.

- Viruses and cells should be injected as sample, not immobilized on the surface. The detection decreases exponentially with the distance from the sensor surface and an interaction on the surface of an immobilized virus or cell may be too distant from the surface to give a detectable response.

## 2.3 Buffer recommendations

A continuous flow of running buffer is maintained over the sensor surface between injections in a run. The recommended running buffer for your assay depends on the type of molecules used in the interaction, which assay will be run and the type of sensor chip used.

For many protein interactions you may use the Biacore HBS-EP+ buffer. This HEPES-based buffer contains the non-ionic detergent Surfactant P20 and a low concentration of EDTA in physiological salt concentration. The surfactant helps to prevent adsorption of proteins to the walls of the flow system and non-specific binding of material to the sensor surface.

### *2.3.1 Buffers for sample preparation*

It is recommended to prepare the samples in the running buffer to avoid bulk effects during the injections due to differences in refractive index between running buffer and sample.

## 2.4 Assay duration

The total assay times depend on the number of steps included and how long dissociation times your interacting molecules have in the assay step. Ligand immobilization takes approximately half an hour and a typical analysis cycle takes 5–15 minutes.

# 3. Basic instrument operation

This chapter describes basic operation of Biacore X100 instrument. It is assumed that the instrument and the PC have been installed as described in Appendix A. For information on how to select assays, optimize assay conditions and perform a run, see Chapter 4.

*WARNING!* *QUALIFIED OPERATION*
**Biacore X100 should only be operated by properly qualified personnel. Read this manual before operating the instrument.**

*WARNING!*
**Wear appropriate protective clothing when handling reagents and samples.**

## 3.1 Starting the instrument

If the instrument is shut down, start according to Section 3.1.1.
If the instrument is in standby mode, start according to Section 3.1.2.

### *3.1.1 Starting the instrument from shutdown*

Note: If a degasser is installed, always place the buffer tubes in liquid when the instrument is powered on to prolong the lifetime of the degasser.

1. Switch on the printer and the PC.

2. Switch on the instrument at the rear.

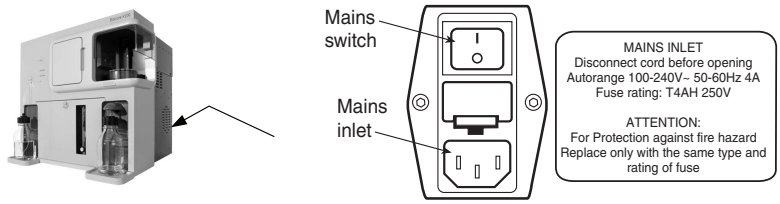



**Figure 3-1**. *Mains input panel at the rear of the instrument.*

The lamps on the front panel light during initiation. When the initiation is ready, **Power** is lit, the **Temperature** lamp is lit or flashes, **Sensor chip** is not lit or flashes and **Run** is not lit.

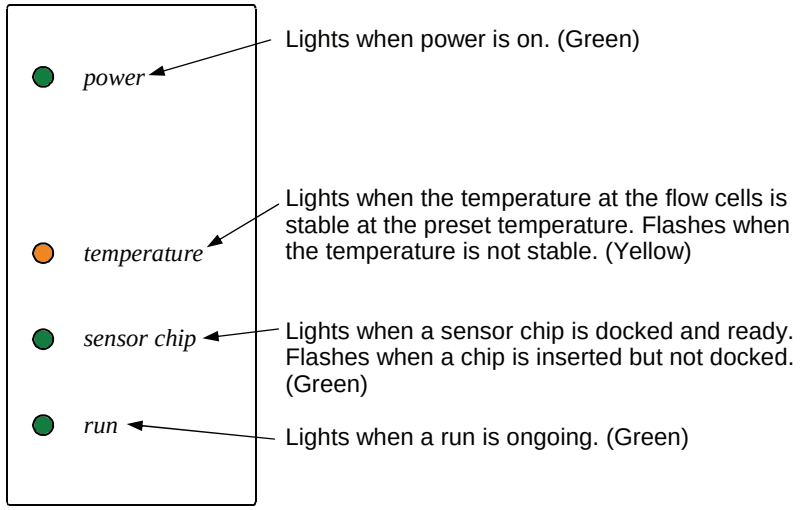



**Figure 3-2**. *Status indicators on the front panel.*

3. Start **Biacore X100 Control Software** from the Windows start menu.

4. In the login-dialog, enter your user name and password and click **OK**. (Your user name and password is provided by your Biacore X100 administrator.)


If you need to select a different database connection, click **Options>>**. The default setup is **This machine**.

5. The software establishes connection with the instrument, which takes about 30 seconds.

6. Ensure that there is fresh buffer or water in the bottle on the left-hand tray and that both buffer tubes are inserted into the bottle. See also Section 3.2.1.

7. Ensure that the waste bottle on the right-hand tray is empty and the waste tube is inserted in the bottle.

8. The system event log is displayed in the software and you are recommended to perform startup. Click **Run now** and follow the instructions on the screen. A previously used chip can be docked for the startup-procedure (see Section 3.3 for instructions on docking a sensor chip).

   Do not forget to close the cover of the peristaltic pump (see Figure 3-3).

**Figure 3-3.** *Closing the cover of the peristaltic pump.*

Once the startup procedure is finished, the instrument is automatically left in standby.

### 3.1.2 Starting the instrument from standby

The instrument is normally left in standby mode which allows new runs to be started quickly.

1. If Biacore X100 Control Software is not started, start it from the Windows start menu.

2. In the login dialog, enter your user name and password and click **OK**. (You get your user name and password from your Biacore X100 administrator.)


If you need to select a different database connection, click **Options>>**. The default setup is **This machine**.

3. The software establishes connection with the instrument, which takes about 30 seconds.

4. Ensure that there is fresh buffer or water in the bottle on the left-hand tray and that both buffer tubes are inserted into the bottle. See also Section 3.2.1.

5. Ensure that the waste bottle on the right-hand tray is empty and the waste tube is inserted in the bottle.

## 3.2 Preparing and loading buffer

### *3.2.1 Buffer preparation*

Select buffer type depending on your specific experiments. For many protein interactions you may use HBS-EP+ buffer supplied by Biacore.

To prepare buffer, dilute 10× buffer solution with degassed deionized water (filtered 0.22 µm). Use degassed water to prevent problems with air bubbles during the run. If the instrument is equipped with the degasser provided with the Biacore X100 Plus Package, you do not need to degas the water.

Always use buffer, fresh for the day and filtered through a 0.22 µm filter to remove particles. A volume of 200 ml is suitable for use during 24 hours.

### 3.2.2 Loading buffer

Prepare buffer in a bottle and place it on the left-hand tray and insert the two buffer tubes. The flow system is filled with buffer automatically when a chip is docked (see Section 3.1 and 3.3).

If the system is in standby, follow the instructions in Section 3.2.3.

### 3.2.3 Changing buffer

If you need to change buffer when the instrument is in standby, stop the standby (**Tools:Stop Standby**) and change the buffer bottle. When both buffer tubes are inserted in the new buffer, select **Tools:Prime** to fill the system with the new buffer.

## 3.3 Inserting, removing or changing the sensor chip


### 3.3.1 Sensor chip and detection

The sensor chip is a gold-coated glass slide mounted on a supporting frame and enclosed in a protective cassette.

> *Caution!*
> Do not take the sensor chip out of its protective cassette. Dust or other particles on the sensor chip surface can seriously interfere with detection.

*Sensor chip types*

Biacore X100 should only be used with sensor chips from Biacore AB. Series S sensor chips cannot be used with Biacore X100.

There are several different types of sensor chips to fit different molecules with different attachment chemistries. A brief overview of sensor chip types is given in Section 2.2.2.

*Detection*

Two flow cells are formed when the sensor chip is docked in the instrument. The interactions on the sensor surface are detected via the optical unit. See Appendix E for a description of the SPR principle and more detailed information of the detection system.

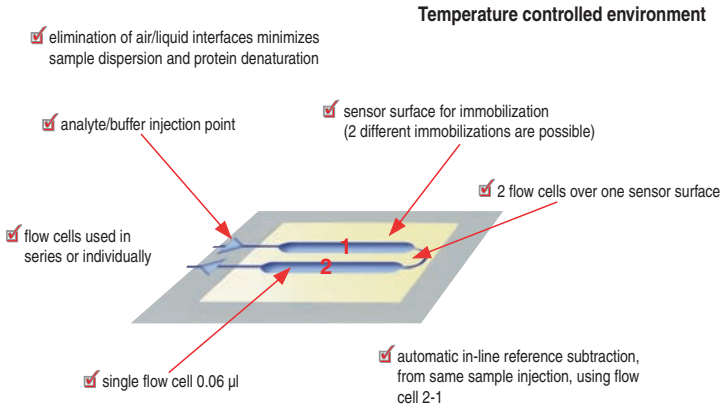



**Figure 3-4.** *Flow cells on the sensor chip.*

### 3.3.2 Inserting a sensor chip

1. Open the upper front door of the instrument.

2. Click the undock chip icon, or select **Undock Chip** from the **Tools** menu. When undocking is completed, the **Dock Chip** dialog is displayed and the sensor chip lamp on the front flashes.

3. Pull out the chip slide, see Figure 3-5 A.

4. If required, remove the old chip, see Figure 3-5 B.

5. Insert the chip with the text on the upper side, see Figure 3-5 C.

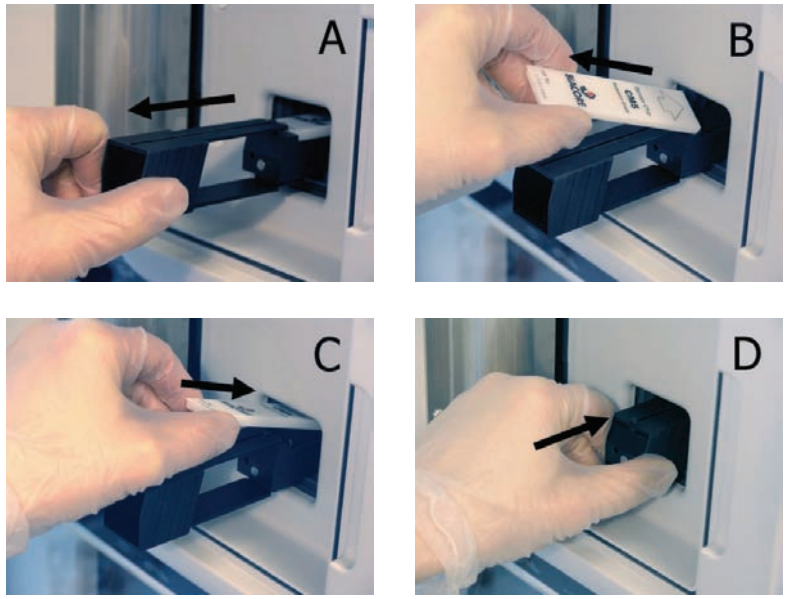



**Figure 3-5**. *Removing and inserting a sensor chip, step A-D.*

6. Push the chip slide all the way in, see Figure 3-5 D.

7. Close the front door and check that the buffer tubes are placed in a bottle of buffer.

8. Fill in the information in the **Dock Chip** dialog and click **Dock Chip**.

The chip is docked and the standby flow of buffer over the chip starts automatically.

## 3.4 Analysis temperature

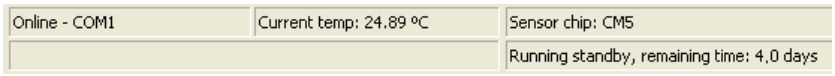
Interaction analysis is sensitive to changes in temperature. It is important that a constant temperature is maintained at the sensor chip surface throughout the run. The detection area housing the sensor chip is maintained at a precisely controlled temperature.

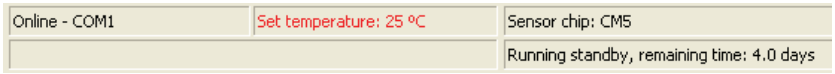
Note: Avoid placing the instrument adjacent to air conditioning or heating units, or in direct sunlight.

The analysis temperature is fixed at 25°C in the standard instrument. With the optional Biacore X100 Plus Package, the analysis temperature can be set to 4-40°C (maximum 10°C below ambient temperature, see Section 6.2).

The analysis temperature is displayed in the status bar at the bottom of the screen.

Unstable temperature is indicated via
- a flashing **Temperature** indicator on the instrument front panel,
- a flashing temperature in the status bar and
- a note in the event log in the Control Software during a run.



## 3.5 Preparing and loading samples and reagents

### *3.5.1 Sample compartment*

*WARNING!* *SHARP NEEDLE*
**The injection needle is sharp. Take care when working in the sample compartment.**

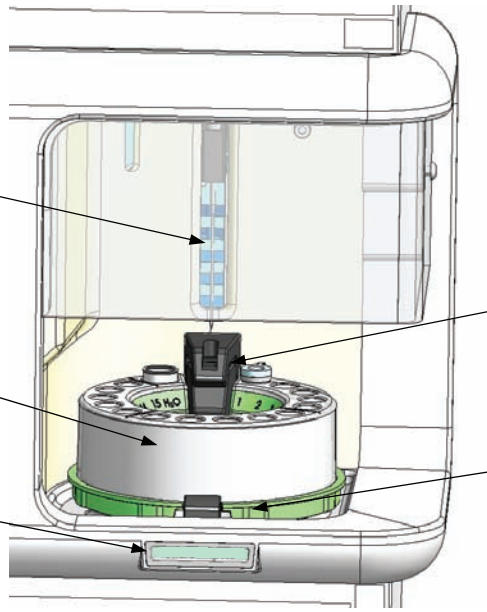
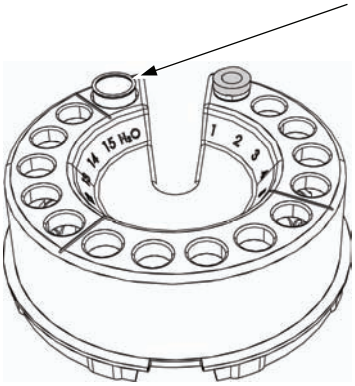


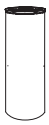

**Figure 3-6**. *The sample compartment.*

*Caution!*
**Do not touch the rack when Rack locked lamp on the front panel is lit (controlled via software). The rack moves occasionally during the run and the needle may be destroyed if the rack is stopped in the movement.**

**Do not attempt to rotate the rack by hand in the instrument.**




**Position $H_2O$:**
One flat-bottomed Biacore 4 ml vial, for deionized water used for needle wash, during run and standby.



NEVER cap!

**Positions 1-15:**
Up to 15 conical Biacore 1.5 ml vials.
For samples and reagents.

Cap with Biacore cap to avoid evaporation during the run.

**Figure 3-7**. *Sample rack with vials.*

> ***Caution!***
> **Use only Biacore vials and caps in the rack. If you use other vials or caps, the needle may be damaged.**

### *3.5.2 Samples and reagents*

Different reagents are used depending on the type of run to be performed. The requirements for your assay are displayed in the rack position list in the Control Software. The list can be printed. If dilution of samples is needed, dilute in degassed running buffer.

The volumes specified in the rack position list are minimum volumes with due consideration for dead volumes.

1. Prepare the samples and reagents in Biacore 1.5 ml conical vials (according to the volumes and concentrations in the rack position list) and cap them with Biacore caps to avoid evaporation during the run. Make sure there are no visible air bubbles in the tubes.

2. Fill the Biacore 4 ml vial with water. Always use fresh water filtered through a 0.22 µm filter to remove particles. Do not cap the 4 ml vial! The water is used for needle cleaning during the run and during standby.

3. Load the rack as described in Section 3.5.3.

### 3.5.3 Loading the rack

1. Click the **Load Samples** icon.

2. Wait until the lamp **Rack locked** is switched off.

3. Lift out the rack and place the sample and reagent vials and the water vial according to the rack position list.

Note: Load the rack outside the instrument.

4. Insert the rack in the sample compartment and make sure it sits properly on the rack base.

5. Click **OK** in the **Load Samples** dialog.

## 3.6 Starting a run

Runs are started from a workflow, via a wizard or via a manual run. See Chapter 4 for more information.

## 3.7 After a run

After a run, take care of the chip (see Section 3.7.1) and choose whether to leave the instrument in standby or perform a shutdown (see Section 3.7.2).

### 3.7.1 Chip handling after a run

| The chip used in the run | Action |
|---|---|
| will be reused in next run | Leave the chip in the instrument and ensure there is enough buffer in the buffer bottle for the standby period. |
| will not be reused in next run but in a later run | Remove the chip and store the chip wet or dry according to recommendations in Biacore Sensor Surface Handbook.<br>Insert an old chip or the maintenance chip and start standby. |
| will not be used again | Leave the chip in the instrument or replace it with the maintenance chip and start standby. |

### 3.7.2 Standby or shutdown

| If next run will be made… | Action |
|---|---|
| within 4 days | Prepare the system for Standby mode, see Section 7.4. |
| later than 4 days | Perform a Shutdown, see Section 7.5. |

# 4. Developing and running assays

## 4.1 Biacore X100 Control Software

With Biacore X100 you can run kinetics/affinity assays, binding assays, various other wizards and manual runs. If you have the optional Biacore X100 Plus Package, you can also perform concentration analysis assays and single-cycle kinetic assays.

Biacore X100 Control Software is used to control the instrument and to evaluate the assay development steps.

This chapter gives an overview of the Control Software and the different assays that can be performed. For detailed information, see the Support Navigator in the software.

### *4.1.1 Logging in to Biacore X100 Control Software*

1. Start Biacore X100 Control Software from the Windows start menu, using **Biacore:Biacore X100 Control Software**.


2. Enter your user name and password and click **OK**.

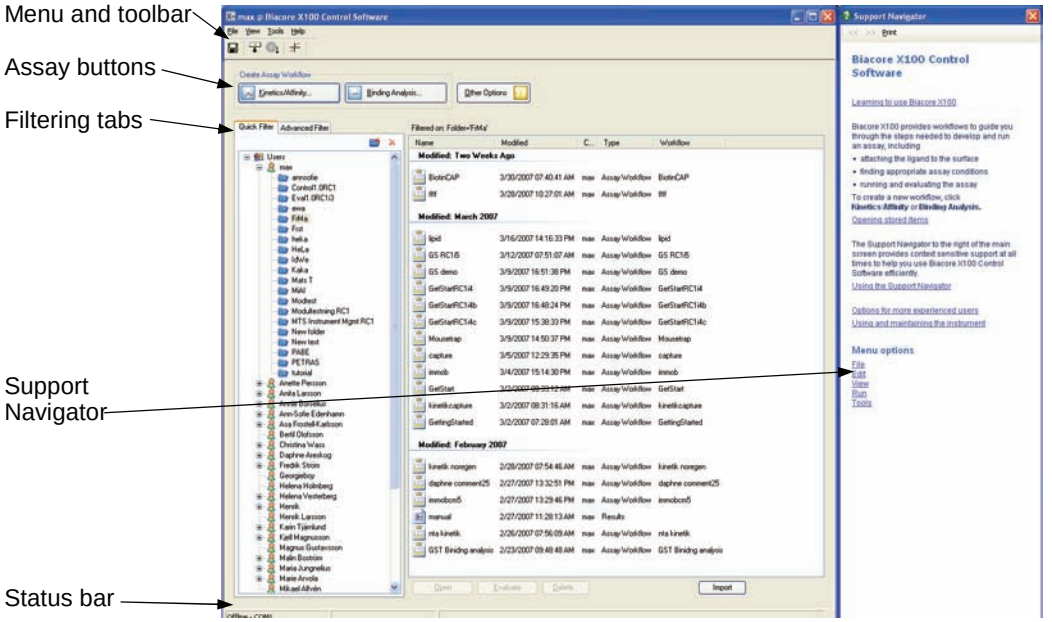
   If you need to select a different database connection, click **Options>>**. The default setup is **This machine**.

Your Biacore X100 administrator can provide you with user and database information. For more detailed database information, see Appendix E.

### 4.1.2 Start view

The start view in the Control Software is by default displayed with the Support Navigator to the right to make it easy for you to find support on all occasions.

The start view contains the following areas and functions:




- The ***menu and toolbar*** provide access to control commands. For details, see the Support Navigator.

- The ***assay buttons*** are used to set up and start your runs. For details, see Section 4.2.

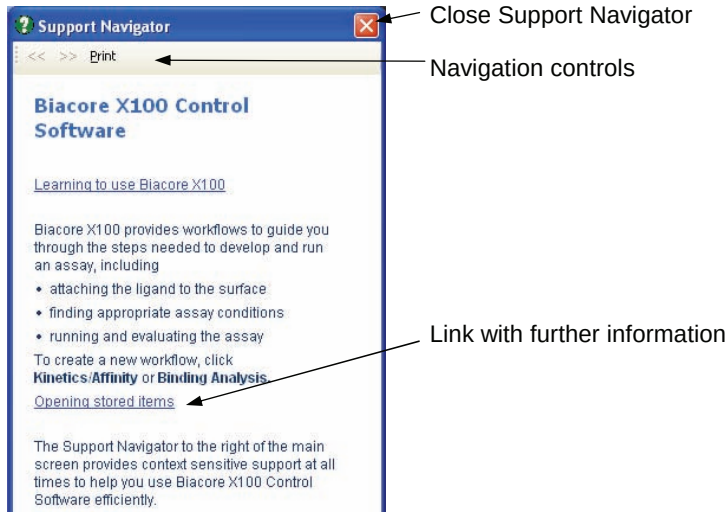
- There are two ***Filter tabs***:
  The ***Quick Filter*** tab displays all users in the database, their different sub-folders in the database and allows for filtering of data via users.
  The ***Advanced Filter*** tab allows for more advanced filtering.
  For details, see Section 4.1.4 and the Support Navigator.

- The ***status bar*** displays the instrument status, including the set and current temperature and the type of chip docked.

- The ***Support Navigator*** displays support for the specific dialog currently displayed. As you move along in the program you will get continuous support to the right. For details, see Section 4.1.3.

### 4.1.3 Support Navigator

The Support Navigator to the right of the main screen provides support synchronized with the software to help you use Biacore X100 Software efficiently at all times. Recommendations, tips and troubleshooting are included in the support. A supplementary web-based support is directly available via links in the software. It can also be accessed via the Biacore website using the product key.




Find more information by clicking on the highlighted links in the Support Navigator.

Retrace your steps using the Back and Forward arrows.

You can close the Support Navigator with the Close button. To open the help again, click **Help** in any dialog or select **Help:Show**.

### 4.1.4 Database contents and display

All data from Biacore X100 is collected in a database and organized in folders. Each user has a folder of their own in which they can create new sub-folders. All users can view, edit and save data in any user's existing folder. Only the user who created the data and Biacore X100 administrators can delete data.

The database can contain four different item types:

| Icon | Item type | Description |
|---|---|---|
|  | Assay Workflow | Contains the selections made for a specific workflow and links to runs already performed.<br>You can open a workflow to see which steps have been performed, redo steps if desired and continue with steps that have not yet been run. |
|  | Wizard Template | Contains the saved settings for a wizard. Can be opened to start a new run if desired. |
|  | Results | Contains the result data for a specific run.<br>Results from assay development steps are evaluated in the Control Software.<br>Results from assay steps are evaluated in the Evaluation Software. |
|  | Evaluation | Contains a saved evaluation session. Evaluation data can be opened in the Evaluation Software directly or via the Control Software. |

*Quick and Advanced Filter tabs*

The start view displays the items currently filtered from the database. The filtering selection is made on the Quick Filter or Advanced Filter tabs.

The items are by default sorted by the date when they were last modified. Click a different column header to sort the data by the contents of the column. Click repeatedly to toggle the sort direction.

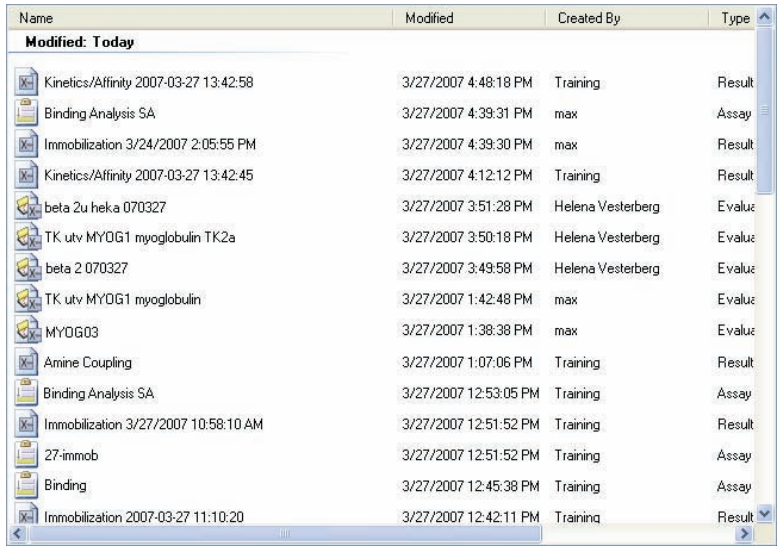



**Figure 4-1.** *Filtered and sorted items.*

The **Quick Filter** tab displays all user folders in the database. Your own folder appears first in the list. Click the **New Folder** icon ( ) to create a new sub-folder. You can only create sub-folders in your own main folder.

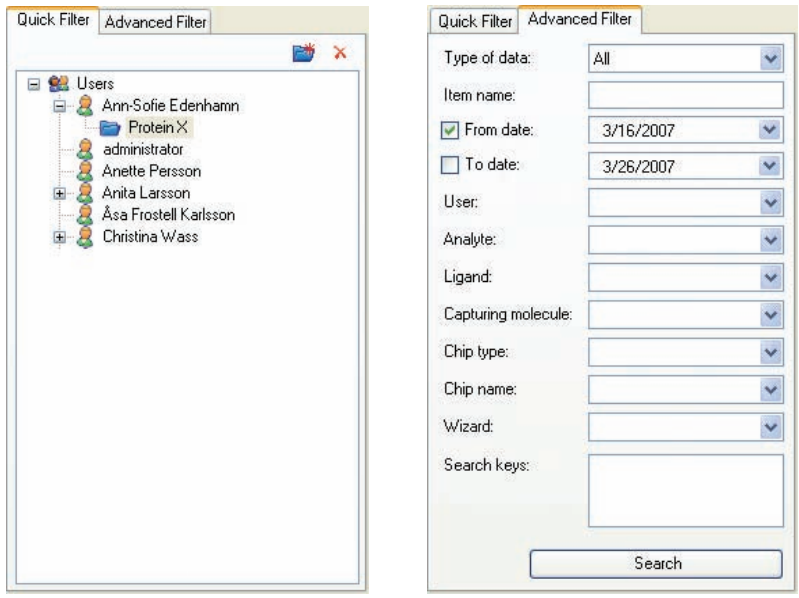



**Figure 4-2**. *All users displayed for a specific Biacore X100 database to the left. The Advanced filtering setting to the right will display all items created March 16, 2007 or later.*

Use the **Advanced Filter** tab to perform more advanced filtering of items in the database. You can search information by entering information in one or several of the boxes on the Advanced Filter tab. You can enter part of the name or the full name or select from the scroll list for each of the boxes you want to use for the search. For searching by dates, click the checkbox to make the filtering active. Once all desired information is entered, click the **Search** button to view all filtered items.

If you want to refine or expand your search, simply continue to change or add information in the boxes and then click the **Search** button again.

*Opening, evaluating or deleting items*
Select the item and then:

- click the **Open** button to open the item in the Control Software.
- click the **Evaluate** button to open the item in the Evaluation Software (possible for Evaluation items and Results items).
- click the **Delete** button to permanently remove the item from the database. Items can only be deleted by their creator or an administrator.

As an alternative, double-click on an item to open it in the Evaluation Software for evaluation items and in the Control Software for other items.

*Exporting and importing items*
Export and import of items can be used to move data between databases and to export data for processing in third-party software.

- To export items for import into another database:
  1. Right-click on the item to export and select **Export:To other database**.
  2. Select where to save the export file, enter a file name and click **Save**.
- To export items for import into other types of programs:
  1. Right-click on the item to export and select **Export:To file**.
  2. Select where to save the export file, enter a file name and click **Save**.

- To import items:

  1. Select the folder into which you want to import the file.

  2. Click the **Import** button.

  3. Browse to the file you want to import (file type either **Biacore X100 Exported File** or **Biacore X100 Exported Assay Workflows**) and click **Open**.

     The item in the file is imported to the selected folder in the database.

## 4.2 Creating assay workflows

### *4.2.1 What is a workflow?*

Biacore X100 provides workflows to guide you through the steps needed to develop and run an assay, including

- attaching the ligand to the surface
- finding appropriate assay conditions
- running and evaluating the assay

A complete assay includes at least preparation of a sensor surface and then running an assay. For some assay formats, sensor surface preparation is performed as part of the assay step. Assay development steps are available to find the best conditions.

Workflows give you recommendations for choice of sensor surface, ligand attachment methods and steps in assay development and execution. The recommendations are based on the user input of ligand details and use reagent kits from Biacore with predefined assay conditions where possible. The workflow helps you to achieve optimal results for your experiment with minimal previous knowledge of the system, and also provides a framework for organizing the work done in relation to a given assay.

Settings that you enter in the workflow will be fixed in assay development and assay steps. You can enter settings either from the results of assay development steps or directly in the workflow if you already know suitable conditions.

Experimental steps in a workflow are run using assay development and assay wizards with settings fixed according to the workflow requirements. The same wizards can also be run outside the context of

a workflow, allowing you to use your own wizard settings as appropriate (see Section 4.3).

Workflows can be set up for kinetics/affinity analysis and general binding analysis experiments. Concentration analysis and custom assay design are supported by wizards in the optional Biacore X100 Plus Package.

### 4.2.2 Overview of steps in a workflow

A workflow will contain some or all of the steps listed below (see Figure 4-4). The steps included in a particular workflow are determined by the assay design and ligand attachment approach chosen for the workflow in the initial selection step. Each workflow step links to a wizard with preset conditions to suit your selected ligand and ligand attachment, minimizing the input needed from you.

Assay development steps provide an experimental basis for determining parameters required in subsequent assay development or assay step. You can run assay development steps any number of times in a given workflow, and save the results of a selected run as the settings for subsequent steps. Alternatively, if you know the conditions you want to use, you can skip the assay development step and enter the settings manually in the workflow. Settings that are entered in a workflow are fixed in the subsequent workflow steps. If no settings are entered in the workflow, you will have to enter settings each time you run the assay.

*Find immobilization pH*

This surface preparation step will help you to determine the optimal pH for covalently immobilizing your ligand or capturing molecule on the sensor surface. If you are using Biacore kits where immobilization conditions are already known, this step will not appear in the workflow.

The immobilization pH is important for efficient attachment of proteins from relatively dilute solutions. Efficient attachment relies on electrostatic preconcentration of protein molecules on the negatively charged sensor surface.

This step is always run on an unmodified sensor chip surface. The sensor chip can be used afterwards for continued work.

*Immobilize*

This surface preparation step immobilizes ligand or capturing molecule on the surface using covalent chemistry or permanent capture on Sensor Chip SA. The step is not included for workflows that rely on reversible ligand capture on ready-to-use sensor chips (Sensor Chip NTA, Sensor Chip L1 and Sensor Chip CAP).

***Find capture conditions***
This assay development step is only valid for capture and will help you to:

- determine whether the capturing interaction is appropriate for the assay
- choose a suitable ligand concentration and contact time for capture

***Find sample conditions***
This assay development step will help you to:

- determine whether the ligand is active (i.e. whether analyte binds or not)
- choose suitable concentrations and contact time for your samples
- estimate the analyte binding capacity of the surface (which is a measure of the activity of the attached ligand)
- determine whether regeneration of the surface is required or whether analyte can be allowed to dissociate spontaneously between cycles
- determine whether there is significant unwanted binding to the reference surface

***Find regeneration conditions***
This assay development step will help you to determine the optimal conditions for regeneration of the surface, by examining the efficiency of regeneration using different conditions. If you are using Biacore kits where regeneration conditions are already known, this step will not appear in the workflow.

***Run assay***
Running this step will start the wizard for the workflow assay, using the settings that you have entered in the workflow.

### 4.2.3 Creating a workflow

For detailed support see the Support Navigator to the right in the software.

1. Click the relevant button to create a workflow for **Kinetics/Affinity** or for **Binding Analysis**.


   In the examples below a Kinetics/Affinity Assay workflow is shown. The dialog for a Binding Analysis Assay workflow is similar.

2. In the **Create Assay Workflow** dialog that opens, enter ligand name and select the type of ligand.


   Depending on your input, you will get recommendations on what chip to use, suitable attachment approaches and what procedures to include in your workflow. Biacore kits and reagents are recommended where possible for a simplified assay workflow with known immobilization pH and/or regeneration conditions.

3. Select the ligand attachment approach, and if required enter a capturing molecule. A preview of the recommended assay workflow for the current selection is displayed to the right.

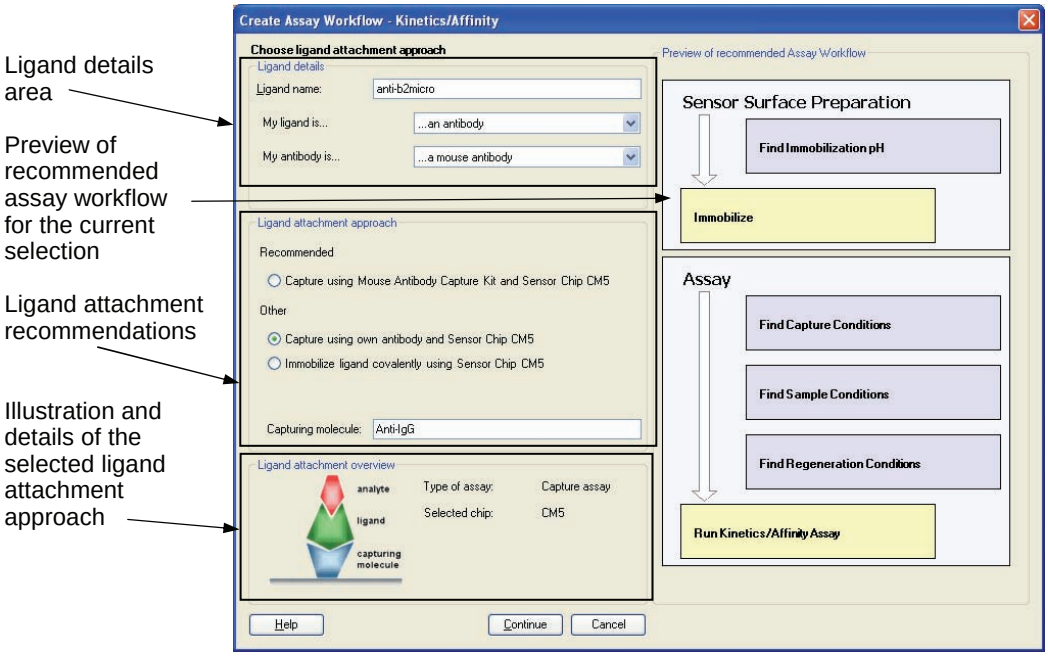



**Figure 4-3**. *Example of the creation of a Kinetics/Affinity workflow with a preview of the recommended assay workflow to the right in the dialog.*

4. Click Continue and save the workflow in one of your folders.

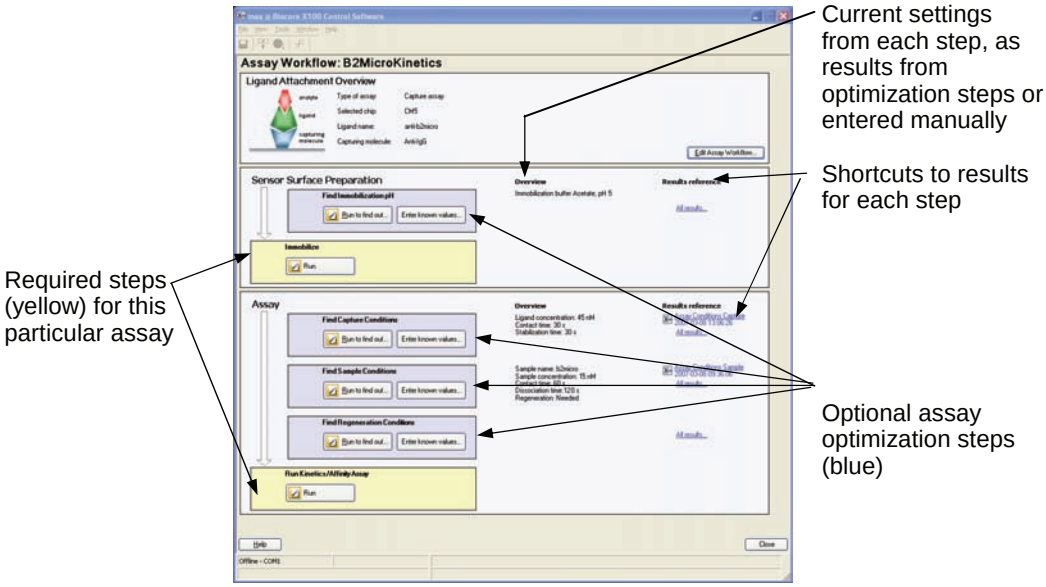



**Figure 4-4**. *Example of a saved Kinetics/Affinity workflow with required steps in yellow boxes and optional assay development steps in blue boxes. Runs are started from the buttons in the boxes.*

5. Continue by running the desired steps from top to bottom (required steps are yellow, optional steps are blue). You can find detailed support for each step in the Support Navigator.

### 4.2.4 Editing a workflow

Before any runs have been started in a workflow, you can edit ligand name, ligand type and ligand attachment approach by clicking the **Edit Assay Workflow** button in the workflow. If any runs have been performed in the workflow, you can view the workflow definition but you cannot edit the settings.

### 4.2.5 Automatic display of result data

The results from the assay run (the last yellow box in a workflow) are displayed automatically in the Evaluation Software. All other results are automatically displayed in the Control Software when the run is finished.

## 4.3 Other ways to run assays on Biacore X100

Apart from workflow assays you can also run assays using the wizards outside a workflow (with less settings locked and no preset order) or using manual runs. See the following sections for more details.

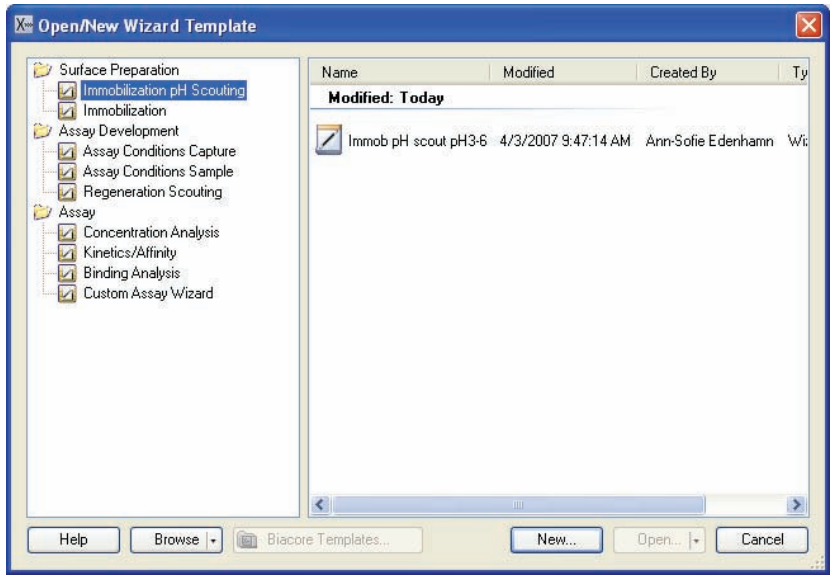
### 4.3.1 Using wizards

Wizards provide direct entry points to specific types of run. Wizards support the same types of experiment as workflows but without the integrated assay overview and automatic transfer of settings to subsequent steps. If you have the Plus Package installed, wizards are also available for concentration analysis and custom-designed assays. Settings for a wizard run can be saved in a wizard template.

| Wizard | Corresponding workflow step |
|---|---|
| Immobilization pH Scouting | Find immobilization pH |
| Immobilization | Immobilization |
| Assay Conditions Capture | Find capture conditions |
| Assay Conditions Sample | Find sample conditions |
| Regeneration Scouting | Find regeneration conditions |
| Concentration Analysis | – |
| Kinetics/Affinity | Assay step, kinetics/affinity workflow |
| Binding Analysis | Assay step, binding analysis workflow |
| Custom Assay Wizard | – |


1. Click **Other Options>>** to expand the choices.


2. To set up a new wizard template or start a wizard run, click **Wizards...**.




3. Double-click on a wizard type in the left panel, or select the wizard type and click **New**, to create a new wizard template of that type.

   The Concentration Analysis wizard and the Custom Assay Wizard are only available if you have the optional Biacore X100 Plus Package installed.

   To open an existing wizard template from the right panel in the dialog, double-click on the template or select the template and click **Open**. You may also click **Browse** or **Biacore Templates** to find more templates. Only templates corresponding to the currently selected wizard type are shown.

4. Continue with the setup and the actual run. Detailed help for each step is provided in the Support Navigator to the right all through the process.

*Automatic display of result data*
Result data from surface preparation wizard runs and assay development wizard runs are automatically displayed in the Control Software when the run is finished. All other wizard results are automatically displayed in the Evaluation Software when the run is finished.

### 4.3.2 Manual runs

There is an option to run the instrument with manual commands to control the run interactively. All settings can be changed during a 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. Use this option for quick interactive experiments such as one-shot yes/no binding tests.

The results from a manual run are saved as a Results item, 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 and binding analysis. It is only possible to view sensorgrams and create plots.


1. Click **Other options>>** to expand the choices.


2. Click **Manual Run...**.to open the **Manual Run** dialog. The option is only available on the system controller.

   Detailed help is provided in the Support Navigator.

# 5. Evaluation

Results of surface preparation and assay development runs are presented automatically in the Control Software when the run is completed. Assay runs are evaluated in the separate Evaluation Software, which offers a range of general functions for evaluation of results:

- Presentation of sensorgrams, report point plots and bar charts
- Assay-specific evaluation for kinetics/affinity and (if the Biacore X100 Plus Package is installed) concentration analysis
- Additional functions for editing result data attributes and presenting results in table format

## 5.1 Starting the Evaluation Software

The Evaluation Software is started automatically when an assay run is completed, or when a result item or a saved evaluation item is opened in the Control Software (see page 30). You will be logged in to the Evaluation Software with the same username as in the Control Software.

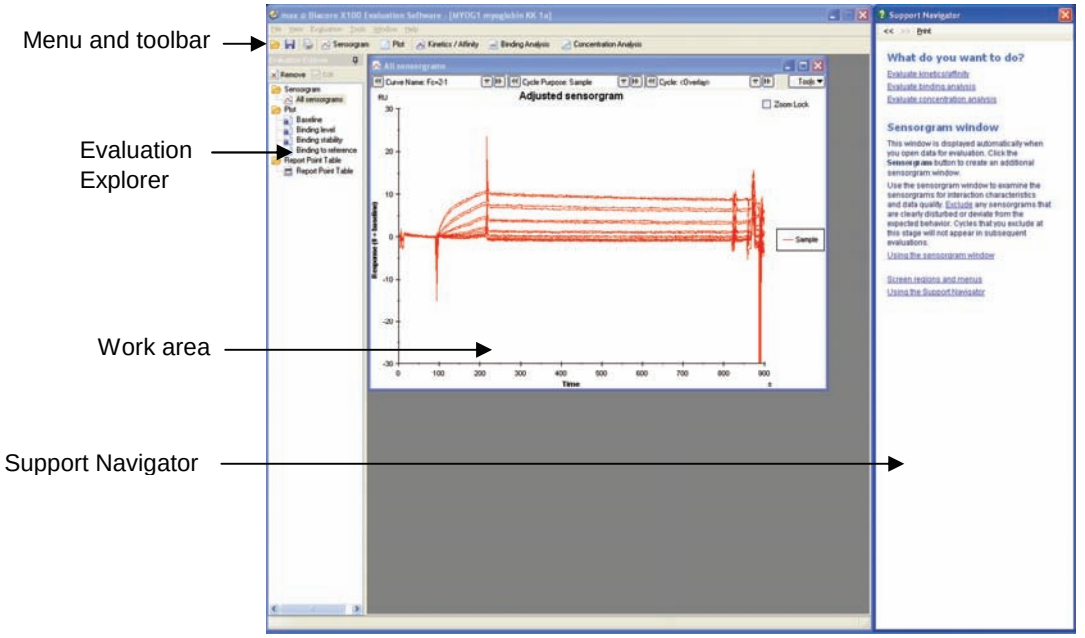
You can open the Evaluation Software independently of the Control Software from the Windows start menu. See Section 4.1.1 for details of the login procedure.

Each evaluation session that is started automatically from the Control Software or manually from the start menu will create a new instance of the Evaluation Software. Close the software when you have finished evaluation to avoid accumulating instances.

## 5.2 Evaluation Software – general features

### 5.2.1 Start view

The start view contains the following areas and functions:




- The ***Menu and toolbar*** provide access to the evaluation functions.
- The ***Evaluation Explorer*** lists the evaluation items (sensorgrams, plots and other results) that have been created in the current session.
- 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 ***Support Navigator*** displays support for the specific dialog currently displayed. As you move along in the program you will get continuous support to the right. For more details, see Section 4.1.3.

### 5.2.2 Opening items

To open data in the Evaluation Software, select **File:Open**. You can open result items and saved evaluation items. Use the Quick Filter or Advanced Filter tab (see Section 4.1.4) to find items.

Opening an item with **File:Open** will automatically close any current evaluation. To combine results from multiple runs in the same session, use the **File:Append Run** option. Appending results to an evaluation session will delete all the user-defined evaluation items in the session.

*Items created during opening*

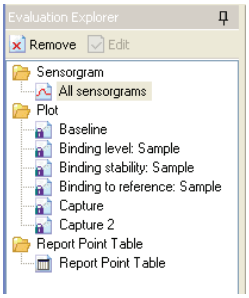
When result data is opened, the results are shown automatically in a **Sensorgram** window. In addition, a set of predefined plots and a report point table are automatically created according to the defined keywords and report points for the result item.

Use the sensorgram window to examine the sensorgrams for interaction characteristics and data quality.

The predefined plots are of different types depending on the assay, e.g. baseline, capture, binding to reference, binding level (see the Support Navigator for a full list and further details). The plots can be used for quality check of the data and troubleshooting as well as for evaluating results.

The definition of keywords and report points for a run also define which evaluations that can be made.

### 5.2.3 The Evaluation explorer




Click on an item in the Evaluation explorer to display it in the work area.

Click the pin icon from  to  to hide the Evaluation explorer panel and give you more screen space for evaluation. The Evaluation explorer will reappear when you move the mouse to the left hand edge of the evaluation window.

Right click in the Evaluation explorer for additional options. Right click on an item for additional options relating to that item.

### 5.2.4 Adding new evaluation items

New evaluation items; sensorgrams, plots, binding analyses and kinetics/affinity analyses; can easily be added by clicking the buttons on the toolbar. If the optional Biacore X100 Plus Package is installed, concentration analysis is also available.


See the Support Navigator for descriptions and detailed information on how to proceed.

### 5.2.5 Right-click menus in the work area

There are several options displayed if you right-click in a window in the work area. 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.

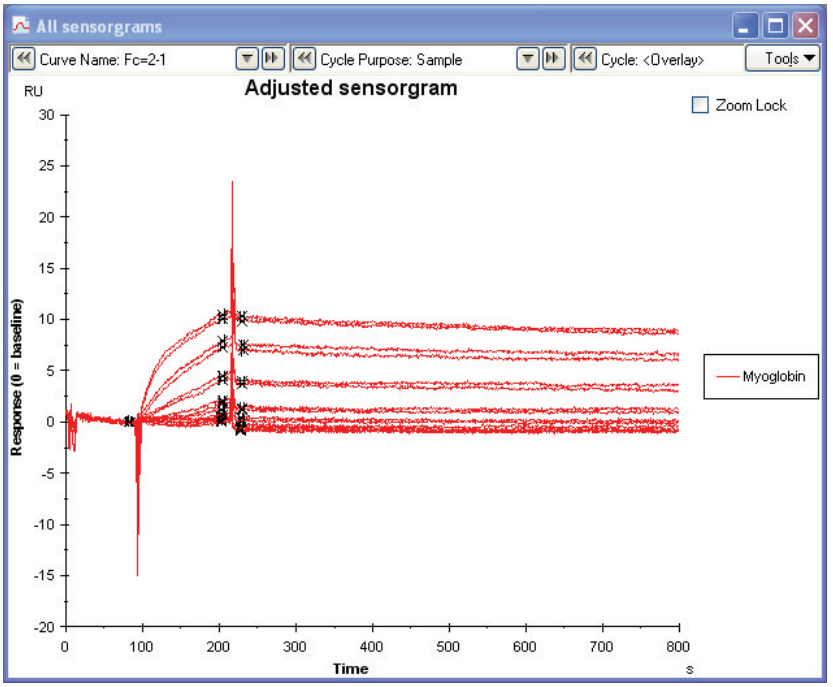
## 5.3 Presenting data

All types of results can be displayed in sensorgram and plot windows and as bar charts using the **Binding Analysis** tool (Section 5.3.3).

This section gives an introductory overview of the presentation functions. More details may be found in the Support Navigator.

### 5.3.1 Sensorgram windows

Sensorgram windows display the results as sensorgrams.



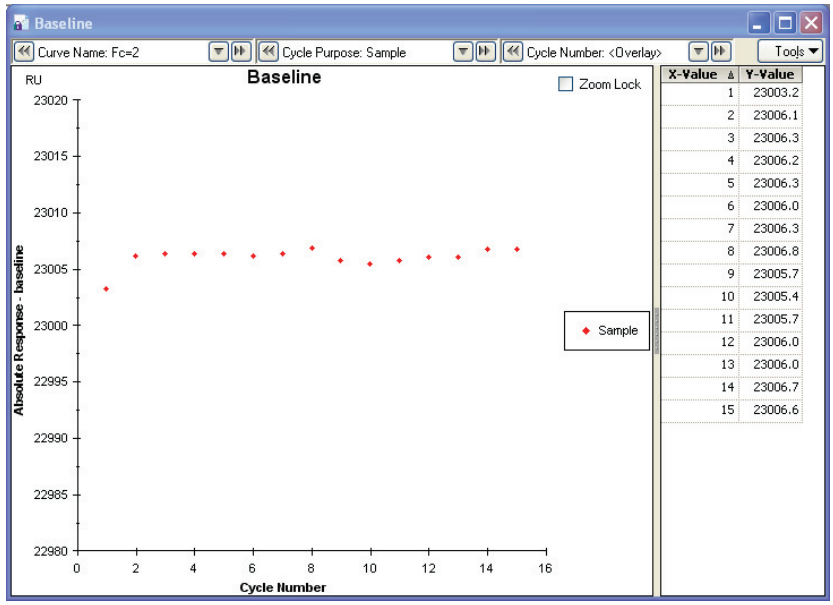

Display functions include

- Select which sensorgrams and cycles to display
- Align sensorgrams to chosen points in the x- and y-directions
- Color sensorgrams according to various properties
- Show report points and event markers in the display

Use the sensorgram window for presentation purposes, and also to examine the data for quality control.

### 5.3.2 Plot windows

Plot windows display scatter plots of report point values against another parameter. Normally, the x-axis in a plot shows cycle number: however, plots can be created using other variables such as cycle purpose or sample name, or using a different report point for the x-axis.



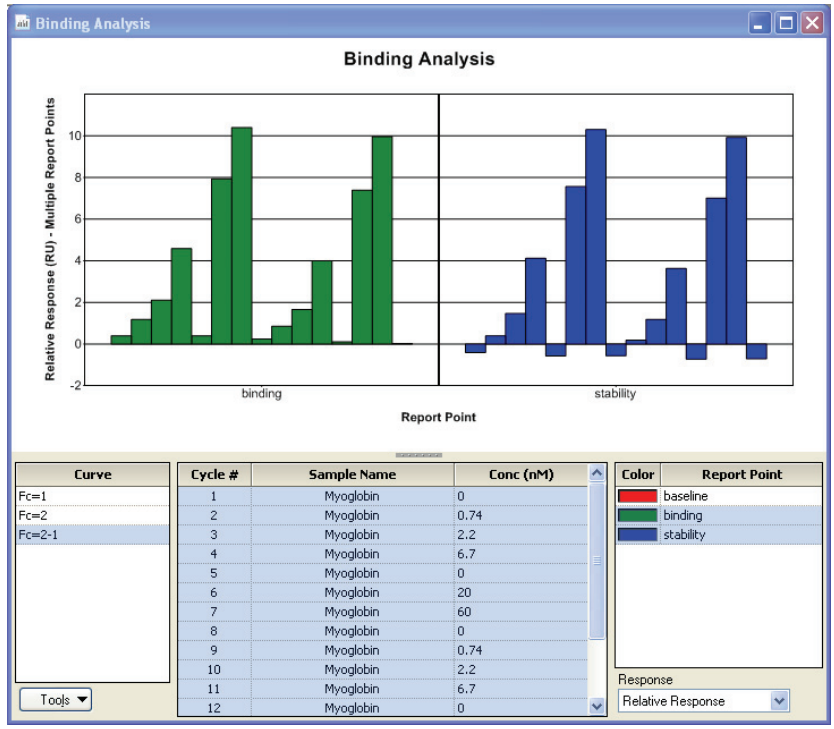

If desired, lines can be fitted to the points in a plot window using either a linear or curved fitting function.

A set of predefined plots is created automatically when results are opened, according to the report points available in the results. These plots are intended in the first place for quality control of the data. See the Support Navigator information for more details of the predefined plots.

Plots can also be used to rank samples in relation to one or two threshold values.

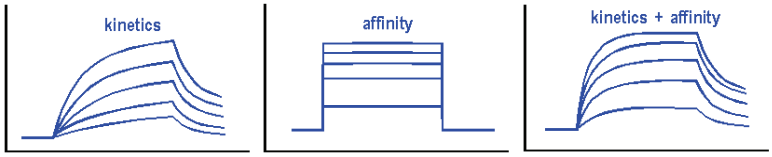
### 5.3.3 Binding analysis

The **Binding Anaysis** tool creates a bar chart presentation of report point values. Bars can be grouped by various criteria, e.g. curves, cycles and report points.



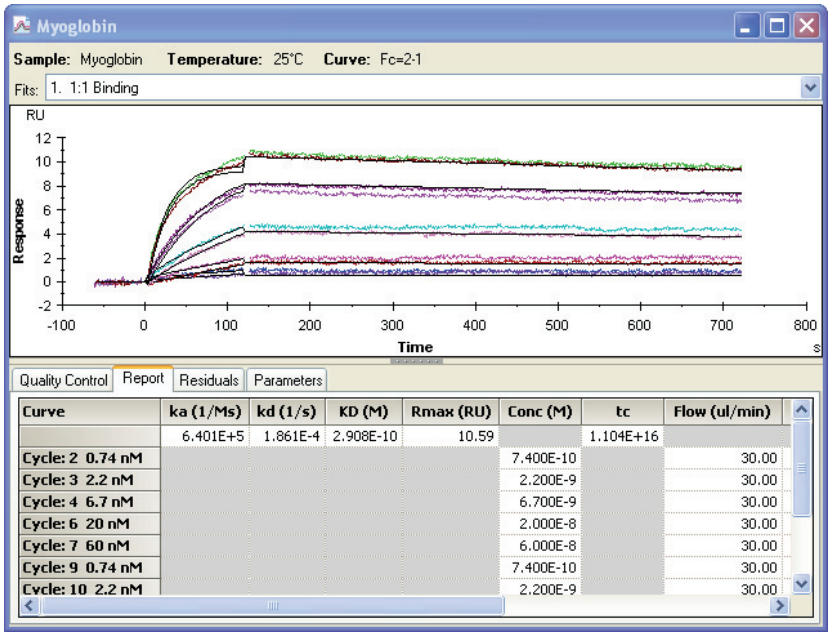
### 5.3.4 Kinetics/Affinity analysis

The **Kinetics/Affinity** tool evaluates sensorgram data in terms of either interaction kinetics or steady state affinity. In general, you can evaluate kinetics for sensorgrams that show sufficient curvature and affinity for sensorgrams that reach steady state during sample injection. In some cases you may be able to apply both evaluations to the same data set.




Kinetics may be evaluated according to a number of different interaction models as described in Appendix D.

Both kinetics and affinity evaluation are performed as a guided series of steps that create a completed evaluation item. For the simplest kinetics model (1:1 interaction), built-in quality control assessment helps you to decide whether the results are reliable. You can edit a completed item if you want to change the settings for the evaluation.

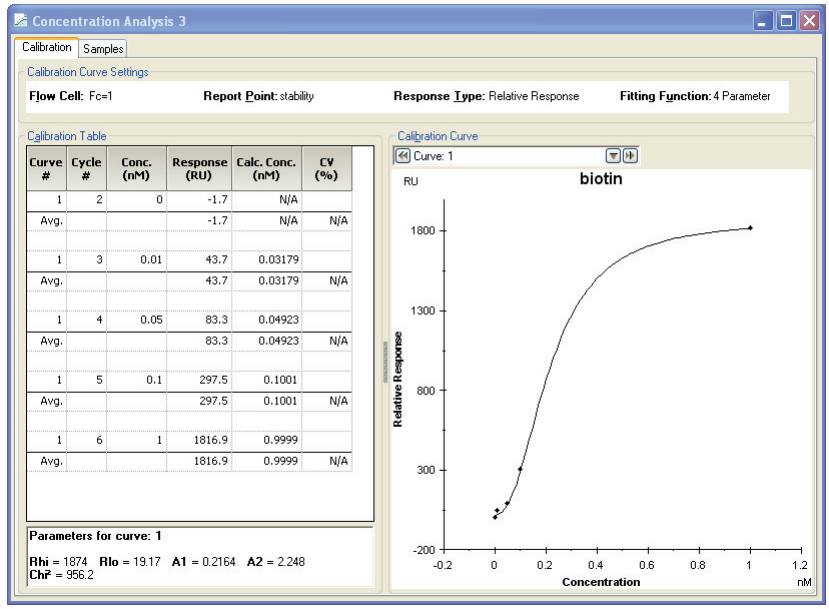



| Curve | ka (1/Ms) | kd (1/s) | KD (M) | Rmax (RU) | Conc (M) | tc | Flow (ul/min) |
|---|---|---|---|---|---|---|---|
| | 6.401E+5 | 1.861E-4 | 2.908E-10 | 10.59 | | 1.104E+16 | |
| Cycle: 2 0.74 nM | | | | | 7.400E-10 | | 30.00 |
| Cycle: 3 2.2 nM | | | | | 2.200E-9 | | 30.00 |
| Cycle: 4 6.7 nM | | | | | 6.700E-9 | | 30.00 |
| Cycle: 6 20 nM | | | | | 2.000E-8 | | 30.00 |
| Cycle: 7 60 nM | | | | | 6.000E-8 | | 30.00 |
| Cycle: 9 0.74 nM | | | | | 7.400E-10 | | 30.00 |
| Cycle: 10 2.2 nM | | | | | 2.200E-9 | | 30.00 |

### 5.3.5 Concentration analysis

The **Concentration Analysis** tool constructs a calibration curve from the response levels in calibration cycles, then calculates analyte concentrations in the sample cycles using the sample response and the calibration curve. Calibration curves may be linear or curved.

This tool is only available if the Biacore X100 Plus Package is installed.



### 5.3.6 Other evaluation functions

*Report point table*
The report point table (created automatically when the results are opened) lists details of all report points and keywords in the results. The table can be exported to a tab-separated text file or a Microsoft Excel file for processing with third-party software if required.

The report point table includes the following statistical functions:

| | |
|---|---|
| **SD** | Standard deviation of data points in the time window, calculated as $SD = \sqrt{\frac{1}{(n-1)}\sum(y_i - \overline{y})^2}$ where n = number of points and y = response in RU |
| **Slope** | Slope during time window in RU $s^{-1}$, calculated as $slope = \frac{\sum\left((y_i - \overline{y})^2(x_i - \overline{x})\right)}{\sum(x_i - \overline{x})^2}$ |
| **LRSD** | Alignment of the slope to a straight line (regression coefficient), calculated as $LRSD = \sqrt{\frac{Q_0}{(n-2)}}$ where $Q_0 = \sum(y_i - \overline{y})^2 - \frac{\left(\sum(y_i - \overline{y})(x_i - \overline{x})\right)^2}{\sum(x_i - \overline{x})^2}$ |

*Custom report points*
A set of report points is created automatically when the run is performed. You can add custom report points to the results if you need additional points for your own customized evaluation.

Choose **Tools:Custom Report Points** from the main menu to create and edit custom report points.

*Keyword table*
Each cycle in a run is associated with a set of predefined keywords that identify properties of the cycle (for example cycle purpose, sample name, sample concentration and so on). These keywords determine which assay-specific evaluation can be applied to the results:

- Multi-cycle kinetics/affinity analysis requires at least one cycle of purpose **Sample** with the same sample name and values for the sample concentration (either in molar units, or in weight units with a value for the molecular weight).

- Single-cycle kinetics analysis requires at least one **Sample** cycle consisting of a series of injections with values for the sample concentrations (either in molar units or in weight units with a value for the molecular weight).

- Concentration analysis requires a minimum number of two cycles of purpose **Calibration** with specified sample concentrations, and at least one cycle of purpose **Sample** where the concentration will be determined. A minimum of 4 calibration points is required for curved (4-parameter) fitting to the calibration curve. The minimum number for linear fitting is 2.

You can edit the values in the predefined keywords, for example to correct data entry errors from the Control Software. You can also add your own keywords for documentation purposes. Custom keywords will not affect kinetics/affinity or concentration analysis evaluation.

Note: If you edit the keyword table, any user-defined evaluation items that you have created will be deleted. Save your evaluation session before editing the keyword table.

## 5.4 Workflow for assay evaluation

Details of assay evaluation may be found in the Support Navigator. This section gives a broad outline of a recommended general workflow for evaluation.

1. Examine the results in the sensorgram window. The shape of the sensorgrams can tell you much about your results, for example what response levels are reached, whether an interaction is fast or slow, whether the results contain kinetic information or only steady state affinity data (Section 5.3.4), whether the sensorgrams are “clean” or are disturbed by spikes, drift or excessive noise and so on.

   Zoom the sensorgram display and use the **Color by** and **Adjust Sensorgrams** functions from the **Tools** button in the sensorgram window to make the display easier to read.

2. If required, clean up your data by excluding any seriously disturbed sensorgrams. Right-click on the sensorgram line and choose **Exclude cycle** to exclude a sensorgram. Cycles that you exclude in the sensorgram window will not be used in subsequent evaluation.

3. If you intend to use report-point based evaluation tools (plot or bar-chart presentations or concentration analysis), choose to show report points in the sensorgram window from the **Tools** button. Check that the report points you are interested in are clear of any sensorgram disturbances and that they are correctly placed. Create custom report points if you need.

4. Examine the predefined plots for assay performance. The plots that are created vary according to the set-up of the assay. Quality assessment criteria depend on the purpose of the assay: see the Support Navigator for details.

5. Perform the assay-specific evaluation. For binding analysis and custom assays, the evaluation tools you should use will depend on how you want to evaluate and present the data. For kinetics/affinity and concentration analysis, use the respective assay-specific tools.

6. Assess your evaluation results in relation to the purpose of the experiment, and repeat or refine the evaluation if required.

7. Save the evaluation session and close the Evaluation Software.

# 6. Biacore X100 Plus Package

The optional Biacore X100 Plus Package is a combined hardware and software package that provides enhanced functionality and flexibility. The oackage includes:

- In-line degasser
- Variable analysis temperature control (4–40° C)
- Custom Assay Wizard for user-defined assays including single-cycle kinetic analysis.
- Concentration Analysis Wizard
- Custom immobilization
- User-defined fitting models for kinetics and affinity

## 6.1 In-line degasser

The in-line buffer degasser eliminates the need to degas running buffer before use. The use of degassed solutions is especially important when running assays at high temperatures.

Two buffer lines pass through independent vacuum chambers in the degasser (see Section C.3). Gas molecules dissolved in the liquid diffuse through membrane tubing in the vacuum chamber.

The vacuum pump of the degasser operates as required as soon as the instrument is powered on. To prolong the lifetime of the degasser, always ensure that there is liquid in the buffer bottle when the instrument is powered on.

## 6.2 Variable analysis temperature control

The temperature control enables analysis in the temperature range 4-40°C (maximum 10° below ambient temperature).

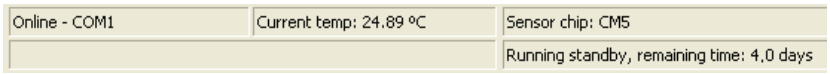
Note: The temperature control applies to the flow cells and does not affect the sample compartment temperature. If you have temperature sensitive samples, keep them on ice for as long as possible before placing them in the rack.

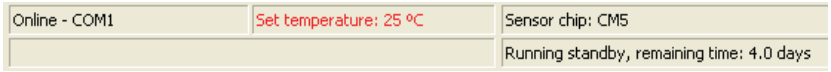
*Setting the analysis temperature*
The temperature is set in the Biacore X100 Control Software. The last set analysis temperature before shutdown is used as default.

1. Select **Tools:Set Temperature** and set the required temperature.

The time required for temperature stabilization depends on the set temperature and the ambient temperature.

2. The set and current temperature are displayed in the status bar.




Once the set temperature is reached, the temperature indicator lights steadily and runs can be started.

## 6.3 Custom assay wizard

By using the Custom Assay Wizard you can define your own assay procedures with high flexibility. You can either change an existing wizard template or create a new one.

### 6.3.1 To create a new wizard template

Click **Other Options>>**, **Wizards,** select **Custom Assay Wizard** and click **New**.

Detailed information on how to select flow cell, chip and evaluation purpose and then continue with the setup is found in the Support Navigator.

*To create a wizard template from an existing template*
1. Click **Other Options>>**, **Wizards** and select **Custom Assay Wizard**.

2. Previously saved templates in the current folder are listed in the right panel of the dialog.

   Use the **Browse** button to find templates exported to disk.

   Click the **Biacore Templates** button to access templates provided by Biacore.

Select the template you wish to use and click **Open**.

3. The template opens with the saved settings displayed in each dialog. Change settings as desired and save the wizard template as a new template. More information is found in the Support Navigator.

### 6.3.2 Single-cycle kinetics

A single-cycle kinetic assay includes a series of sample injections (from low to high concentration) in one cycle, with a dissociation period after the last injection. The surface is not regenerated between injections.

Run separate cycles for blanks, replicates or analysis of different samples.

The method is useful for determining kinetics for interaction systems that are difficult to regenerate. When ligand capture is used, consumption of ligand is minimized since one capture injection is used for all sample concentrations.

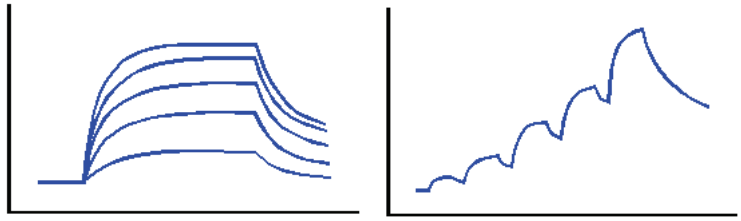


**Figure 6-1.** *Comparison of sensorgrams from multi-cycle kinetic assay (left) and single-cycle kinetic assay (right).*

*To set up a single-cycle kinetics assay*

1. Click **Other Options>>**, **Wizards** and select the **Custom Assay Wizard**.

2. Click **Biacore Templates**, select the template **Single cycle kinetics** and click **Open**. The template is prepared with suggested default settings. Change the settings as required.

   Note: You must keep the evaluation purpose setting as **Kinetics/Affinity** in order to run single-cycle kinetics. The **Single cycle kinetics** option is only available for the **Sample** command when this purpose is selected.

## 6.4 Concentration analysis wizard

Use this wizard to measure analyte concentrations in samples, using known sample to create a calibration curve.

To set up a concentration analysis assay, click **Other Options>>**, **Wizards** and select **Concentration Analysis**. Detailed support is found in the Support Navigator.

## 6.5 Custom immobilization

If none of the predefined custom immobilization methods is appropriate for your application, you can create custom immobilization methods.

1. In Biacore X100 Control Software, click **Other options>>**, **Wizards** and select **Immobilization**.

2. Click **Custom Methods** to open the **Custom Methods** dialog.

For more information, see the Support Navigator in the software.

## 6.6 User-defined fitting models for kinetics and affinity

You can create user defined fitting models if the Biacore X100 Plus Package is installed. Click **Tools:Models** and select **Kinetics** or **Affinity**. For more information, see Appendix D in this handbook and the Support Navigator in the software.

# 7. Maintenance

## 7.1 Introduction

This chapter summarizes user maintenance and describes the software tools for user maintenance procedures. If more extensive service of the instrument is required, please contact your local Service representative.

Make sure that the BIAmaintenance Kit is available before starting maintenance procedures.

*WARNING!*

- Always wear appropriate protective clothing during operation and maintenance.
- Use required safety equipment when handling hazardous substances.
- If the instrument is contaminated with biohazards, please contact your local Service representative for further information about decontamination procedures.

***Caution!***
Some maintenance procedures will destroy the ligand on a prepared sensor chip. Always use the separate Sensor Chip Maintenance that is included in the maintenance kit unless otherwise stated.

Do not use BIAdesorb solution 1 at analysis temperatures below 20 °C. BIAdesorb solution 1 precipitates at low temperatures.

## 7.2 Maintenance summary

Regular maintenance of Biacore X100 is essential for reliable results. It is important to keep the instrument free from contamination such as microbial growth and adsorbed proteins in the liquid handling system.

Regular checks and maintenance should be done according to the schedules below. You will be reminded of the need for **Desorb**, **Desorb and Sanitize** and **System Check and Pump Calibration** procedures via the Control Software, displayed in the System Event log. **Do not ignore maintenance reminders for more than a day or so!**

To display the System Event log, click **View:System Event log**.

### 7.2.1 User maintenance operations

| Interval | Action | Required material |
|---|---|---|
| **Daily/after each run** | Empty the waste bottle. | - |
| **Weekly** | Clean the flow system.*<br>Insert a Sensor Chip Maintenance, select **Tools:More Tools:Desorb** and follow the instructions on screens. | BIAdesorb solution 1 and 2, Sensor Chip Maintenance, deionized water (filtered 0.22 µm) |
| | Inspect tube fittings and pumps, check for leaks. See Section 7.3.1. | - |
| **Monthly** | Clean and disinfect the flow system.<br>Insert a Sensor Chip Maintenance, select **Tools:More Tools:Desorb and Sanitize** and follow the instructions on screens. | BIAdesorb solution 1 and 2, BIAdisinfectant solution, Sensor Chip Maintenance, deionized water (filtered 0.22 µm) |
| | Perform a **System Check and Pump Calibration.**<br>Insert a new Sensor Chip CM5, select **Tools:More Tools:System Check and Pump Calibration** and follow the instructions on screens. | BIAtest solution, HBS-EP+ buffer, a new Sensor Chip CM5 |
| | Inspect the needle and needle wash station, look for accumulations of salt or protein. | - |
| | Clean the instrument cover. See Section 7.3.2. | Deionized water (filtered 0.22 µm), mild detergent, lint-free cloths |

**Table 7-1.** *Schedule for user maintenance operations.*

* For some applications, a coating of protein on the tubing and IFC channel walls helps to give reproducible results, and the first few cycles after Desorb may be unreliable. If you observe this behavior, run Desorb when you change your protein system rather than once a week.

### 7.2.2 Cleaning the needle and wash station

Clean the needle and wash station if you see that salt or other deposits have accumulated. You will need deionized water, lint-free tissues, and an Allen key to remove shield (supplied with the instrument).

Run **Tools:More Tools:Clean needle and needle wash station** and follow the instructions on the screen.

### 7.2.3 Preventive maintenance

To ensure correct performance of Biacore X100, a qualified local Service representative should perform preventive maintenance at regular intervals. During the maintenance visit, worn parts are replaced and all vital modules of the Biacore X100 system are tested.

## 7.3 User maintenance operations

All user maintenance operations are displayed in Table 7-1. Details not covered in the table can be found below.

### *7.3.1 Checking for leaks*

The pump compartment is placed behind the lower front door. Once a week, check that there are no liquid or salt deposits at the following positions:

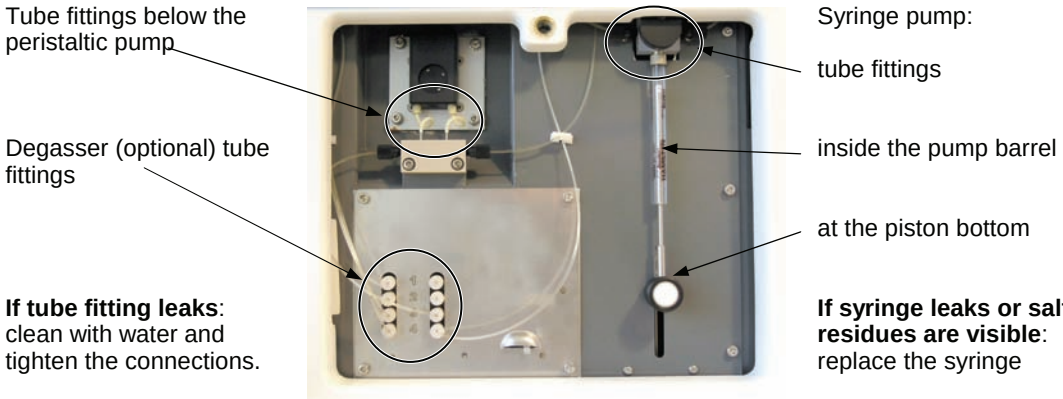



**Figure 7-1**. *Positions to check for leakage.*

### *7.3.2 Cleaning the instrument cover*

If necessary, clean the cover of the instrument with a moist cloth. Use water or a mild detergent. The buffer tray and the waste tray can be removed for cleaning. If salt residues or proteins have accumulated on the buffer bottle cap, clean the cap in deionized water.

## 7.4 Standby

Biacore X100 is automatically placed in standby mode, for a maximum of 4 days, after the end of each run. In standby mode the instrument maintains a continuous low flow of buffer or water from the left hand bottle through the flow system to prevent accumulation of buffer residues and to preserve the ligand on the chip.

If you intend to leave the instrument for up to 4 days, check that you have:

- a chip docked in the instrument
- at least 120 ml buffer or water in the buffer bottle with buffer tubes inserted
- a full water vial in the rack (The water is used for regular washes of

the needle to minimize salt deposits.)
• an empty waste bottle.

You may restart the standby again after 4 days, after checking the above list.

Notes: We recommend that you use distilled and filtered water instead of buffer to minimize salt deposits. However, if an immobilized sensor chip is docked and will be used later, buffer may be necessary to preserve the sensor surface during standby.

During the standby period you can leave the buffer in the instrument for up to 4 days. However, if you start a new run, change to a buffer fresh for the day.

*Starting standby manually*
To start the standby, select **Tools:Standby** in the Control Software.
If you want to close the software but leave the instrument in standby, select **File:Exit** and in the dialog that is displayed, select **Standby** and click **Next**.

## 7.5 Shutdown

If the instrument will not be used within 4 days or if the instrument will be moved, perform a shutdown.

It is recommended to perform a **Desorb and sanitize** procedure (see Section 7.2.1) and, if required, also a **Clean needle and needle wash station** procedure (see Section 8.1) before starting the shutdown procedure.

The shutdown procedure flushes the flow system first with distilled water, then with 70% ethanol and finally with air to dry the system.

*Required material:*
• Deionized water (filtered 0.22 µm)
• 70% ethanol

*Shutting down the instrument*
1. Prepare 2 bottles: one with distilled and filtered water and the other one with 70% ethanol.

2. Choose **File:Exit** and then select **Shutdown** or choose **Tools:Shutdown**.
   Follow the instructions on the screen.

The procedure flushes the flow system and then empties the IFC of liquid. The total run time is about 10 minutes.

When instructed, open the cover of the peristaltic pump to release the clamping of the pump tube, see Figure 7-2

**Figure 7-2.** *Opening the cover of the peristaltic pump to release the clamping of the pump tube. The cover is found behind the lower front door.*

3. Exit from Biacore X100 Control Software if it is still running.

4. Remove the bottles, and the buffer bottle cap assembly. Clean the bottle trays if required. Insert the buffer tubes in an empty bottle.

   The chip is undocked during the *Shutdown* procedure. An undocked chip is indicated with a flashing sensor chip lamp.

   You may remove the chip if desired: pull out the chip slide behind the upper front door and lift out the chip.

# 8. In case of problems

This chapter gives a brief guide of how to solve instrument-related problems that might occur when using Biacore X100.

To avoid problems, follow the instructions and recommendations given in this Handbook, and clean and maintain the instrument regularly as described in Chapter 7. If you are experiencing problems that you cannot correct, contact your local Service representative.

## 8.1 Instrument related problems

See if your problem is described in ***Table 8-2***. If not, continue your search further on in this section.

| Problem | Action | More information |
|---|---|---|
| Proteins and other substances adsorb to the walls of the flow system, giving carry-over problems in assays. | Clean the flow system with **Desorb** more often than once a week. | Section 7.2. |
| Salt residues or other deposits have accumulated on the needle or needle wash station. | Clean the needle and wash station | Section 7.2. |
| Unexpectedly low sample consumption, irregular sensorgrams | Run **Tools:More Tools:Flow system wash**. | – |
| Carry-over problems persist after Desorb and Sanitize. | Run **Tools:More Tools:Superclean**. | Section 8.1.4. |
| The needle is obstructed or damaged. | Replace the needle (**Tools:More Tools:Replace Needle**). | Section 8.1.1. |
| The System Check and Pump Calibration show incorrect volume delivery from the peristaltic pump. | Replace the peristaltic pump tube (**Tools:More Tools:Replace Peristaltic Pump Tube**). | – |
| The syringe leaks or salt residues accumulate below the syringe piston. | Replace the pump syringe (**Tools:More Tools:Replace Syringe**). | – |

**Table 8-2.** *Instrument related problems and corrective actions.*

### 8.1.1 Damaged needle

| Possible cause of needle damage: | Actions |
|---|---|
| Wrong caps have been used on the vials. | Ensure that only Biacore caps are used to cap the 1.5 ml sample and reagent vials. Never cap the water vial.<br>Change needle as described below. |
| Wrong vials have been used. | Ensure that only recommended vials are used, see Section B.4.<br>Change needle as described below. |
| You touched the rack even though the **Rack locked** lamp was lit. | Change needle as described below. |
| The rack mechanism does not function properly. | Contact your local Service representative. |

1. To replace the needle, select **Tools:More Tools:Replace Needle** and follow the instructions on screen.

2. When the needle replacement and calibration is finished, start the standby by clicking **Tools:Standby**.

### 8.1.2 System check and pump calibration

Before contacting your local service representative, run the **System Check and Pump Calibration** tool. The results of this procedure can help your service representative to identify problems. In some cases you may be able to correct the problem yourself.

*To perform a system check and pump calibration procedure:*

1. Insert a new Sensor Chip CM5.

2. Choose **Tools:More Tools:System Check and Pump Calibration** . Follow the instructions on the screen to prepare and start the procedure.

3. The results from the procedure are displayed automatically after the run. Test results are marked as PASS or FAIL.
   If any test failed, perform the action(s) according to the table below and then repeat the System Check and Pump Calibration.
   If the problem persists, contact your local Service representative.

### *If System Check and Pump Calibration fail:*

| Failed test | Actions |
|---|---|
| **A: Peristaltic pump calibration** | Check that the pump cover is properly closed.<br>Check that the buffer tubes reach the buffer liquid and that they are not squeezed.<br>Check that the pump tube does not leak nor has been punctuated by mistake.<br>If a new Peristaltic pump calibration still fails, change the pump tube. |
| **B: Response** | If the Fc-values in the results are too low, check that you used a non-immobilized Sensor Chip CM5.<br>If the Fc-values in the result are too low or too high, check that you used the correct buffer (HBS-EP+ buffer) and test solution (BIAtest solution). |
| **C: Injections** | Check that the tubes do not leak.<br>Contact your local Service representative. |
| **D: Noise** | Check that you used a non-immobilized Sensor Chip CM5.<br>Check that the temperature stability is not affected by inadequate ventilation, sun exposure or similar. |

**Table 8-3** *Actions for different fails in a System check and pump calibration.*

### 8.1.3 Liquid flow problems

| Problem | Possible cause | Action |
|---|---|---|
| Buffer is not supplied properly. | There is not enough buffer in the buffer bottle. | Ensure there is enough buffer before starting the run. |
| | The cover of the peristaltic pump is not properly closed. | Close the cover. |
| | There is a leak in the system. | Check for leaks, see Section 7.3.1. |
| Injection of air during sample or reagent injections. | Insufficient volumes of sample and/or reagents. | Ensure that you use the volumes specified in the software for the run. Larger volumes may be needed if the problem persists.<br>Ensure that you use the recommended caps for sample and reagent vials. Evaporation from uncovered vials will both affect the concentration and reduce the volume.<br>Perform a System Check and Pump Calibration to calibrate the peristaltic pump. |
| Contamination | Not enough water to clean the injection needle. | Always fill the water vial in the rack before starting the run. |

### 8.1.4 Superclean

Use **Superclean** if the regular cleaning procedures are not sufficient.

The following solutions are required for the Superclean procedure.

1. Deionized water (filtered 0.22 µm) at 40–50 °C
2. 1% acetic acid
3. 0.2 M sodium bicarbonate
4. 6 M guanidine-HCl
5. 10 mM HCl

Note: For small molecules, use 50% DMSO and 10% DMSO as the last two solutions respectively.

### 8.1.5 Temperature problems

If the analysis temperature is unstable (the temperature lamp flashes) for more than one hour after the temperature has been set, check that:

- there is at least 20 cm clearance around the instrument to allow adequate circulation.
- the instrument is not placed adjacent to heaters, cooling ducts, or in direct sunlight.

### 8.1.6 Other instrument related problems

Unexpected detection results may appear if there are dust or other particles on the sensor chip surface. Never remove the sensor chip from its protective cassette.

### 8.1.7 Software problems

In the case of software malfunction or other software problem, run **Tools:More Tools:Software Problem Report**. Complete the problem report in as much detail as you can, save the report and submit it by e-mail to your local service representative. Attach any other information you think may be relevant such as screendumps or exported database items. Details of your computer, operating system and installed software are automatically included in the report.

### 8.1.8 For further help

If you experience instrument-related problems and are unable to identify and/or correct them, run **System Check and Pump Calibration** (see Section 8.1.2), then contact your local Service representative.

## 8.2 Assay related problems

The Support Navigator in the software provides help on assay related problems.

# A. Installation requirements

## A.1 General guidelines

Biacore X100 will be installed by a local Service representative. The site should be prepared for the installation as described in Sections A.2 to A.4. If you need to move the instrument after installation, follow the instructions in Section A.5.

## A.2 Space requirements

The size of the instrument is indicated in Figure A-1. At least 20 cm clearance is required on all sides of the instrument to allow adequate air circulation. Space is also required for the PC beside the instrument.

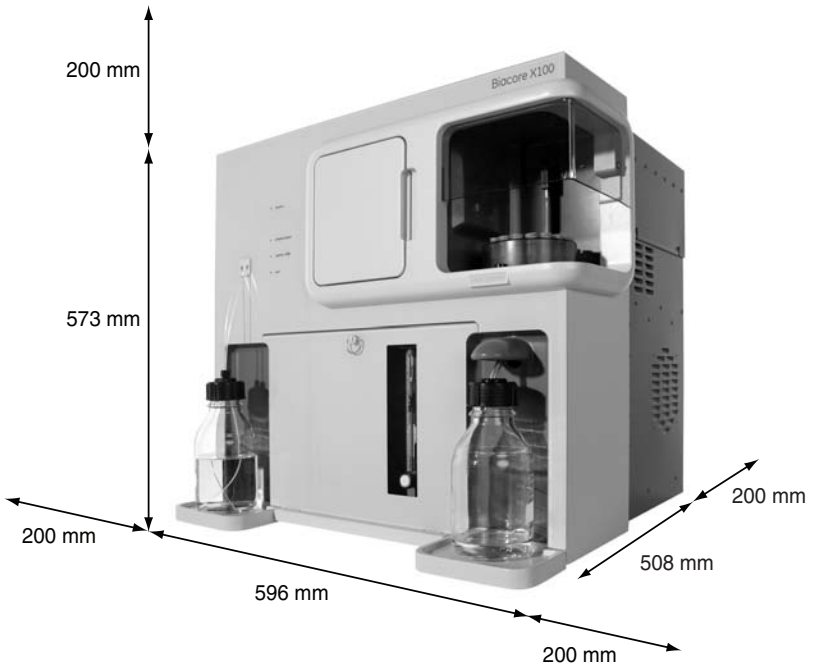



**Figure A-1.** *Space requirements for Biacore X100 instrument.*

| |
|---|
| ***WARNING!*** ***ACCESS TO POWER SWITCH*** |
| Do not block the rear panel of the system. The mains power switch must always be easily accessible. |

## A.3 Mains power supply

The instrument and the PC with printer require mains power outlets with protective earth as specified in Table A-1.

| | |
|---|---|
| Mains voltage | 100-240 Vac (autorange), 50-60 Hz |
| Power consumption: | |
| instrument | maximum 4 A |
| PC and monitor | see manufacturer's manual |
| printer | see manufacturer's manual |

**Table A-1.** *Mains power requirements.*

## A.4 Heating and ventilation

For proper operation, Biacore X100 requires an ambient temperature of 18-33°C and a relative humidity below 80%. Make sure there is adequate air circulation around the instrument.

Note: Avoid placing the system adjacent to heaters, air-conditioner, or in direct sunlight.

## A.5 Moving the instrument within the lab

Note: If the instrument is to be moved more than very limited distances e.g. within a lab, the move should be carried out by a local Service representative.

*WARNING!* *HEAVY OBJECT*
**Biacore X100 weighs 47 kg. Do not try to lift the instrument on your own.**

### *A.5.1 Shutting down the system*

Before moving the system within the lab:

1. Shutdown the instrument completely (See Section 7.5).
2. Shutdown the computer.
3. Disconnect the serial communication cable.
4. Carefully lift the instrument in the metal chassis and place it on a trolley for the relocation.

5. Re-install the instrument in a location that complies with the requirements in Sections A.2 to A.4.

### A.5.2 Connecting to mains power

> ***Caution!***
> Do not turn on the mains power switches before all connections are made.

1. Connect the mains power cord delivered with the instrument, to the **MAINS INLET** connector on the rear panel. Connect the other end to a mains outlet with protective earth.

2. Check that any mains voltage selectors on the PC and peripheral equipment are set correctly.

3. Install the PC and peripheral equipment according to the respective instruction manuals.

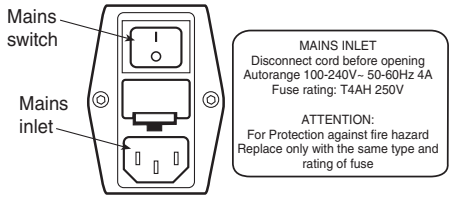



**Figure A-2**. *Mains panel at the rear of the instrument.*

### A.5.3 Connecting the instrument to the PC

Connect a serial communication cable between the **COM1** (or **IOIOIA**) port of the PC, and the **PC** connector on the rear panel of the instrument.

The **SERVICE** connector is only used during instrument service and is then connected to **COM2** on the computer.

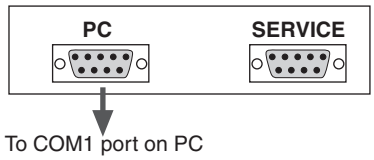



**Figure A-3.** *Computer ports on the rear of the instrument.*

# B. Specifications

## B.1 General

| | |
|---|---|
| Automation | Maximum 15 samples, 24h unattended operation |
| Molecular weight detection | Down to 200 Da in various sample environments |
| Required sample volume | Injection volume + 20-30 µl (application dependent) |
| Sample/reagent capacity | 15 sample/reagent vials, Biacore plastic vials, volume 1.5 ml<br><br>1 water vial, Biacore plastic vial, volume 4.0 ml |
| Analysis time per sample | Typically 5-15 min |
| Detection limit | Typically 0.1 nM for >10 000 Da analytes.<br>Typically 1 nM for <10 000 Da analytes. |
| Kinetics:<br>Association rate constant ($k_a$):<br>Dissociation rate constant ($k_d$): | <br>$10^3$-$10^7$ $M^{-1}s^{-1}$ (for typical protein-protein interactions)<br>$10^{-5}$-$0.1s^{-1}$ |
| Number of flow cells | 2 |
| Flow cell height | Approximately 0.05 mm |
| Flow cell volume | Approximately 0.06 µl |

## B.2 Instrument

| | |
|---|---|
| Dimensions (see also Fig. A-1)<br>W×D×H | 596 × 593 × 573 mm |
| Net weight | Total: 47 kg |
| Mains requirements | Processing Unit and System Controller: Autorange 100–240 V ac, 50–60 Hz, protective earthing |
| Fuses | 2×T4.0AH 250V |
| Power consumption | Processing Unit: max. 4 A (at 100 Vac) |
| Ambient temperature range | 18-33°C |
| Ambient humidity | ≤80 %RH |
| Analysis temperature range | 4-40°C (maximum 10 degrees below ambient temperature) with Biacore X100 Plus Package.<br><br>Without Biacore X100 Plus Package: 25°C. |
| Safety standard, EMC | Complies with and applies to Europe and North America (US and Can) standards, see page 8. |

## B.3 System controller and software

| | |
|---|---|
| PC operating system | OS: Microsoft® Windows® XP (Professional), database storage |
| Power consumption | See manufacturer's manual |

## B.4 Sample rack

| | |
|---|---|
| Capacity | 15 sample vials + 1 water vial |
| Sample/reagent vials | 1.5 ml, conical bottom, with cap |
| Water vial | 4 ml, flat bottom, no cap |

## B.5 Liquid containers provided

| | |
|---|---|
| Running buffer | 500 ml, screw cap |
| Waste | 500 ml, screw cap |

## B.6 Chemical resistance

This section gives some general guidelines concerning chemical resistance for Biacore X100 components.

The flow system and sensor chip are the only parts of Biacore X100 that come into contact with solutions. The guidelines in this section relate to tubing and connectors, selector valves, connector block, IFC and sensor chip.

Note: In most situations, the molecule attached to the sensor surface limits the chemical resistance of the system as a whole.

In general, the flow system components withstand long-term exposure to common aqueous buffer solutions used in biochemical laboratories. Table D-2 below lists compatibility with other common substances.

Concentrated organic solvents as well as long-term exposure to extremes of pH (<3 and >11) should be avoided. For solutions with *short-term* compatibility, do not use as running buffer or for injections longer than 10 minutes. Solutions classed as *long-term* compatible may be used as running buffer.

| Solution | Concentration | Compatibility |
|---|---|---|
| Acetonitrile | 50% | Short term |
| Dimethyl formamide (DMF) | 50% | Short-term |
| Dimethyl sulphoxide (DMSO) | 50%<br>10% | Short-term<br>Long-term |
| Ethanol | 70%<br>10% | Short-term<br>Long-term |
| Ethylene glycol | 100% | Short-term |
| Formic acid | 70% | Short-term |
| Formamide | 40% | Long-term |

**Table B-1.** *Chemical resistance.*

# C. Technical description

This appendix gives a brief description of technical aspects of Biacore X100.

## C.1 Detection principle

### *C.1.1 Surface plasmon resonance*

Biacore X100 exploits the phenomenon of surface plasmon resonance (SPR) to detect and measure analyte. SPR is a phenomenon that occurs in thin conducting films at an interface between media of different refractive index.

Under conditions of total internal reflection, the light leaks an electric field intensity called an *evanescent wave field* across the interface into the medium of lower refractive index, without actually losing net energy.

The amplitude of the evanescent field wave decreases exponentially with distance from the surface, and the effective penetration depth is about half the wavelength of the incident light.

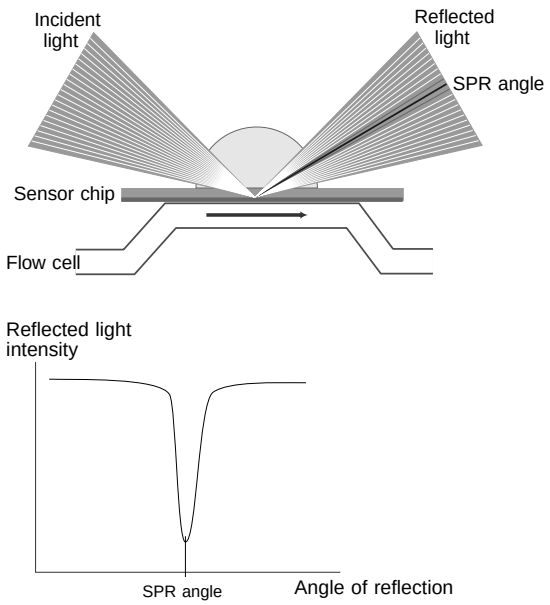



**Figure C-1.** *The SPR principle.*

At a certain combination of angle of incidence and energy (wavelength), the incident light excites *plasmons* (electron charge density waves) in the gold film. As a result, a characteristic absorption of energy via the evanescent wave field occurs and SPR is seen as a drop in the intensity of the reflected light (Figure C-1).

Because the evanescent wave field penetrates the solution, conditions for this resonance effect are very sensitive to the refractive index of the solution within the effective penetration depth of the evanescent field. Changes in solute concentration at the surface of the sensor chip cause changes in the refractive index of the solution, which can be measured as changes in the SPR conditions.

**Note:** The reduced intensity of reflected light is not caused by light absorption in the sample in the conventional (transmission spectroscopy) sense. The light used in Biacore X100 is totally internally reflected inside the optical unit, and it is the evanescent wave that penetrates the sample. Consequently, measurements may be made on turbid or even opaque solutions, without interference from conventional light absorption or scattering by the sample.

### *C.1.2 What SPR measures*

In the configuration used in Biacore X100, the SPR response is a measure of the refractive index of the solution within the penetration distance of the evanescent field wave. This distance is small (about 300 nm) in relation to the volume of sample used, so that effectively SPR measures the refractive index at the surface of the sensor chip.

The refractive index of the solution varies with the solute content. When the detecting molecule is attached to the sensor chip or when analyte binds to the detecting molecule, the solute concentration at the sensor chip surface increases, leading to a change in the SPR signal.

The response measured in Biacore X100 is related to the mass of analyte bound and is largely independent of the nature of the analyte. Refractive index contributions for different solutes are additive, so that the amount of detecting molecule attached and the amount of analyte bound can both be measured with the same detection principle.

## C.2 Detection system

The detection system consists of the sensor chip and the optical unit. Molecules that bind to the sensor chip surface affect the reflection of light from a light source within the optical unit. See Section C.1 for a description of the SPR principle.

The interaction occurs on the surface of the sensor chip, which forms one wall of the flow cell when the sensor chip is docked on to the IFC.

### C.2.1 Sensor chip

The sensor chip is a gold-coated glass slide mounted on a supporting frame. The sensor chip is normally enclosed in a protective cassette. For illustration purposes, Figure C-2 shows the sensor chip removed from the cassette.

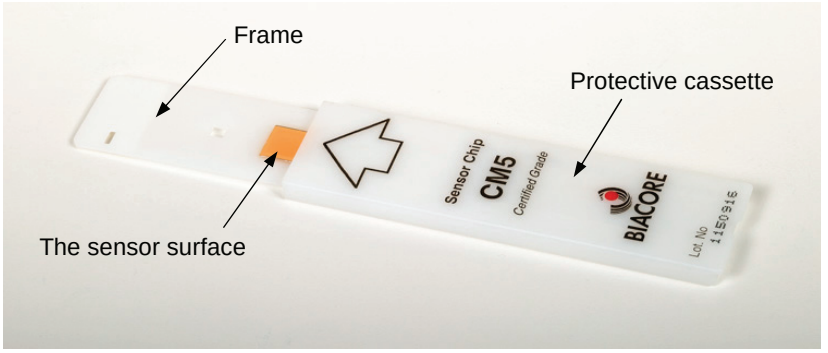



**Figure C-2.** The sensor chip and the protective cassette.

Note: Do not remove the sensor chip from the protective cassette.

The interaction occurs in the surface matrix, which forms one wall of the flow cell when the sensor chip is docked onto the IFC (see below).

### C.2.2 Integrated µ-Fluidic Cartridge (IFC)

The IFC consists of a series of micro channels and membrane valves encased in a plastic housing, and serves to control delivery of liquid to the sensor chip surface.

Samples and reagents are transferred from the sample rack through the needle and into the IFC, which connects directly with the detector flow cells. The sensor chip pressing against molded channels in the IFC forms the flow cells.

Effluent from the flow cells is directed to waste. The IFC is washed automatically as required during a run.

### C.2.3 Flow cells

Two separate flow cells are formed when the sensor chip is docked against the IFC. Precision-cast channels in the surface of the IFC define the flow cells on the sensor chip surface.

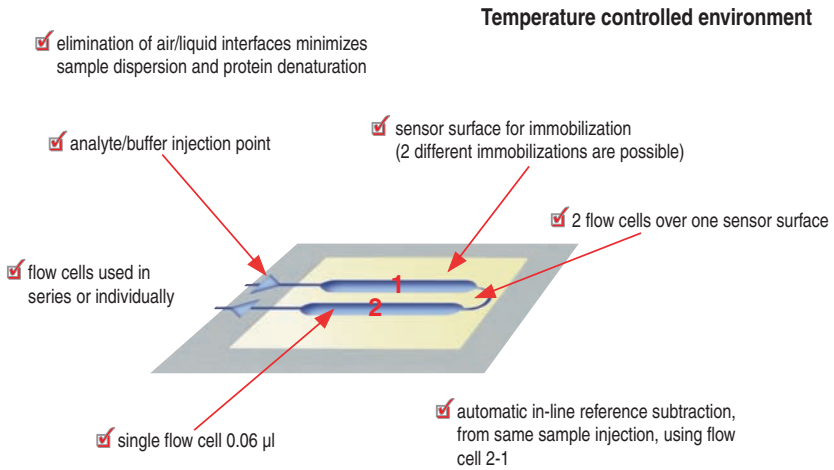



**Figure C-3.** *Flow cells on the sensor chip.*

## C.3 Pump compartment

The pump compartment is placed behind the lower front door The peristaltic pump has a cover that keeps the pump tube in place and clamped when closed.

> ***Caution!***
> Do not place anything on the lower front door when it is open.

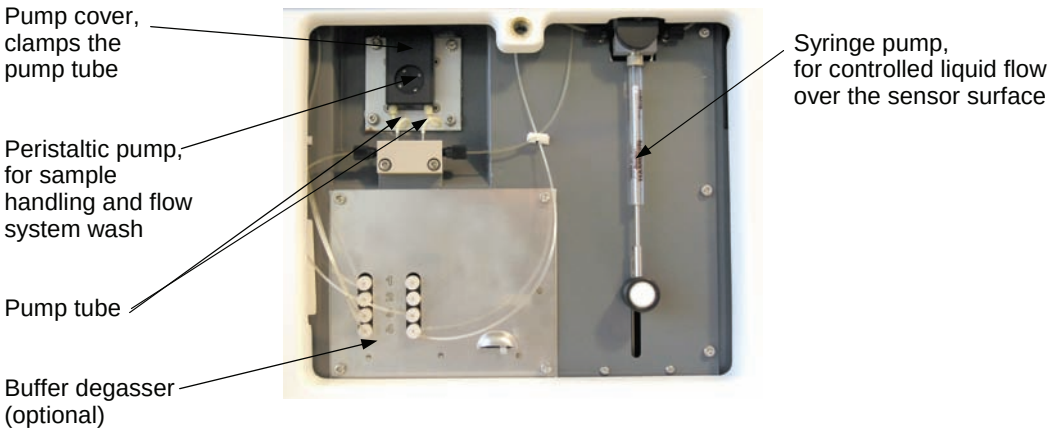



**Figure C-4**. *The pump compartment behind the pump compartment door.*

# D. Kinetic evaluation

## D.1 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.

### *D.1.1 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):

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

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.

### D.1.2 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, 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.

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).

### D.1.3 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, U-value and standard error (SE) or T-value.

***U-value***

The U-value is an estimate of the *uniqueness* of the calculated values for rate constants and $R_{max}$. If parameters are correlated, the fitting procedure can determine their relative magnitudes but not absolute values (for example, knowing the affinity gives the ratio but not the values for rate constants). The U-value is determined by testing the dependence of the fit on correlated variations in pairs of parameters, and is reported as a single value for the whole fitting. U-values above 25 indicate that absolute values for two or more of the parameters (rate constants and $R_{max}$) are correlated and cannot be determined. If the U-value is below about 15 the parameter values are not significantly correlated.

U-values are only determined for fitting to the 1:1 interaction model.

***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.

The ***T-value*** is defined as the parameter value divided by the standard error, and simplifies comparison of significance between parameters with widely different absolute values (e.g. $k_a$ and $k_d$). 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 in 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.

## D.2 Predefined models

A set of predefined models for kinetics and steady state affinity, representing different interaction scenarios, is provided with Biacore X100 Evaluation Software.

*Mass transfer parameters*
All kinetic models include a term describing the rate of mass transfer of analyte from bulk solution to the surface. Since interaction only occurs at the surface, low mass transfer rates may partially or wholly limit the observed binding rate. All models take account of this potential limitation and can extract rate constants from the data provided that mass transfer is not totally limiting. Binding data which is wholly limited by mass transfer does not contain any kinetic information, although valid affinity constants may often be determined.

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 X100 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:

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

### D.2.1 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

The following parameters are fitted by the model:

| | |
|---|---|
| ka | Association rate constant ($M^{-1}s^{-1}$) |
| kd | Dissociation rate constant ($s^{-1}$) |
| Rmax | Analyte binding capacity of the surface (RU) |
| tc | Flow rate-independent part of the mass transfer constant |
| RI | Bulk refractive index contribution in the sample |

### D.2.2 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. As a result of binding of one analyte molecule to two ligand sites, the overall binding is strengthened compared with single-site binding. This effect is 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.

The following parameters are fitted by the model:

| | |
|---|---|
| ka1 | Association rate constant for the first site ($M^{-1}s^{-1}$) |
| kd1 | Dissociation rate constant for the first site ($s^{-1}$) |
| ka2 | Association rate constant for the second site ($RU^{-1}s^{-1}$) |
| kd2 | Dissociation rate constant for the second site ($s^{-1}$) |
| Rmax | Analyte binding capacity of the surface (RU) |
| tc | Flow rate-independent part of the mass transfer constant |
| RI | Bulk refractive index contribution in the sample |

### D.2.3 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

The following parameters are fitted by the model:

| | |
|---|---|
| ka1<br>ka2 | Association rate constant for the first and second analytes ($M^{-1}s^{-1}$) |
| kd1<br>kd2 | Dissociation rate constant for the first and second analytes ($s^{-1}$) |
| tc1<br>tc2 | Flow rate-independent component of the mass transfer constant for the first and second analytes |
| Rmax1<br>Rmax2 | Analyte binding capacity of the surface for the first and second analyte (RU) |
| rcf | Response correction factor relating the contributions of the two analytes. This factor is defined as (Rmax1/Rmax2)/(MW1/MW2). |
| RI | Bulk refractive index contribution in the sample |

### D.2.4 Kinetics – Heterogeneous Ligand

This model describes an interaction between one analyte and two independent ligands (or one ligand with two independent binding sites). 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 ligand sites because the fitting algorithm tends to become unstable with more components, and three or more ligand species cannot be reliably resolved.

The following parameters are fitted by the model:

| | |
|---|---|
| ka1<br>ka2 | Association rate constant for the first and second ligands ($M^{-1}s^{-1}$) |
| kd1<br>kd2 | Dissociation rate constant for the first and second ligands ($s^{-1}$) |
| Rmax1<br>Rmax2 | Analyte binding capacity of the first and second ligands (RU) |
| tc | Flow rate-independent part of the mass transfer constant |
| RI | Bulk refractive index contribution in the sample |

### *D.2.5 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.

The following parameters are fitted by the model:

| | |
|---|---|
| ka1 | Association rate constant for analyte binding ($M^{-1}s^{-1}$) |
| kd1 | Dissociation rate constant for analyte from the complex ($s^{-1}$) |
| ka2 | Forward rate constant for the conformational change ($s^{-1}$) |
| kd2 | Reverse rate constant for the conformational change ($s^{-1}$) |
| Rmax | Analyte binding capacity of the surface (RU) |
| tc | Flow rate-independent part of the mass transfer constant |
| RI | Bulk refractive index contribution in the sample |

### D.2.6 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.

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

The following parameters are fitted by the model:

| | |
|---|---|
| KD | Equilibrium dissociation constant (M) |
| Rmax | Analyte binding capacity of the surface (RU) |
| RI | Bulk refractive index contribution in the sample |

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.

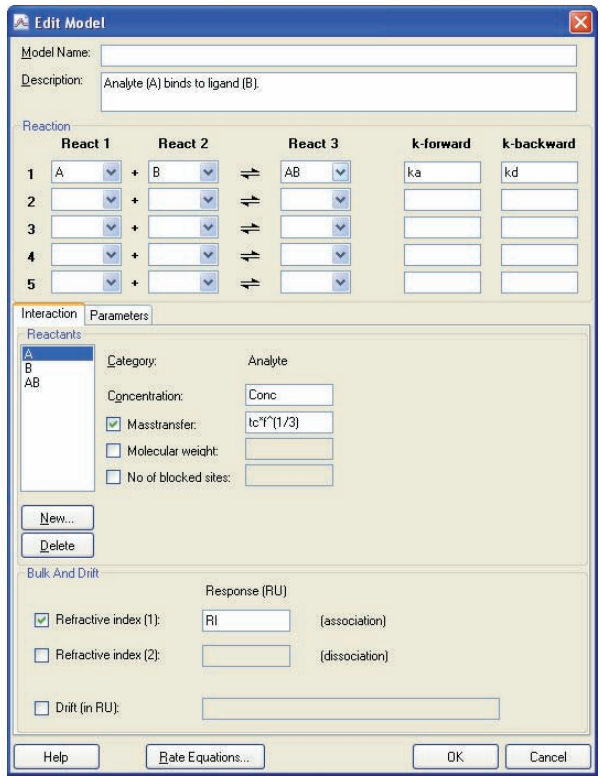
## D.3 Creating and editing models

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 D.3.1 and equation models in Section D.3.2.

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.

### D.3.1 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.




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. |

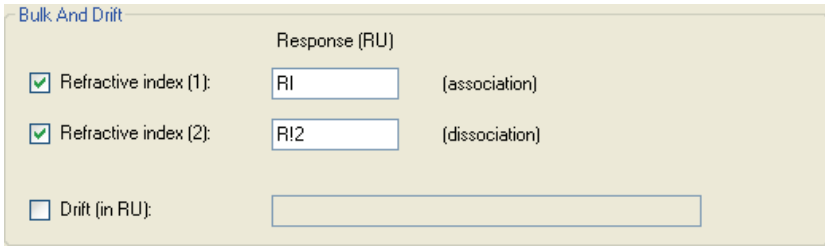
*Ligand*
The ligand is immobilized or captured on the surface, and has the properties listed below. Ligand is usually denoted by the letter B.

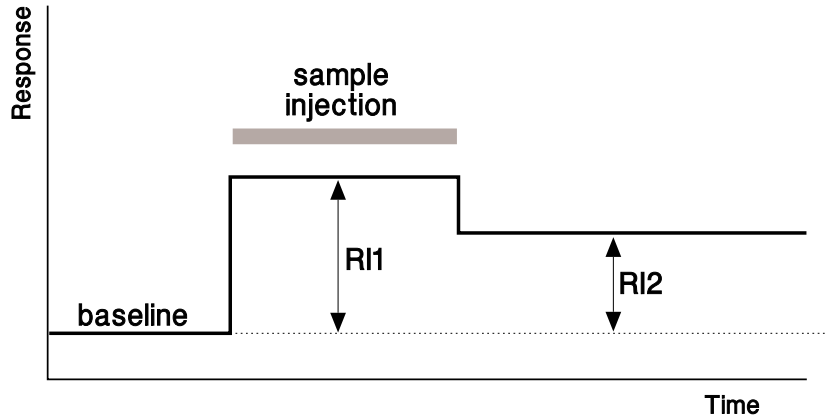
| | |
|---|---|
| **Binding capacity** | Maximum analyte binding capacity of the surface in RU. |
| **At molecular weight** | This parameters is only used in heterogeneous analyte models. Check the box and enter the molecular weight parameter for the analyte to which the binding capacity parameter refers. 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.<br><br>Do not check this box if **Generates response** is also checked. |

1. 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:




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.

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

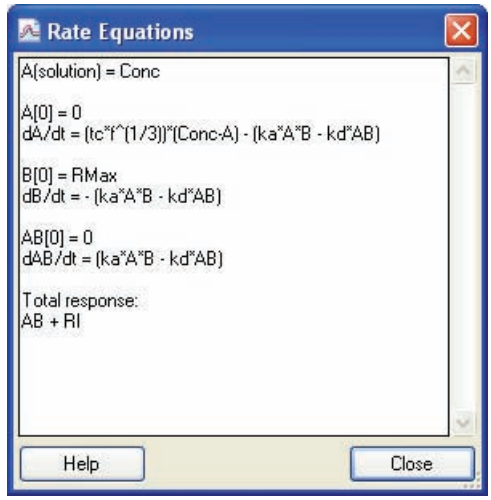
   For the **Initial value**, enter a numerical value or select a value expression from the pull-down list. The expression represent functions evaluated within the current data set (e.g. **Ymax** is the maximum y-value in the data set). Alternatively, choose **Attach to** and select a parameter from the 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.

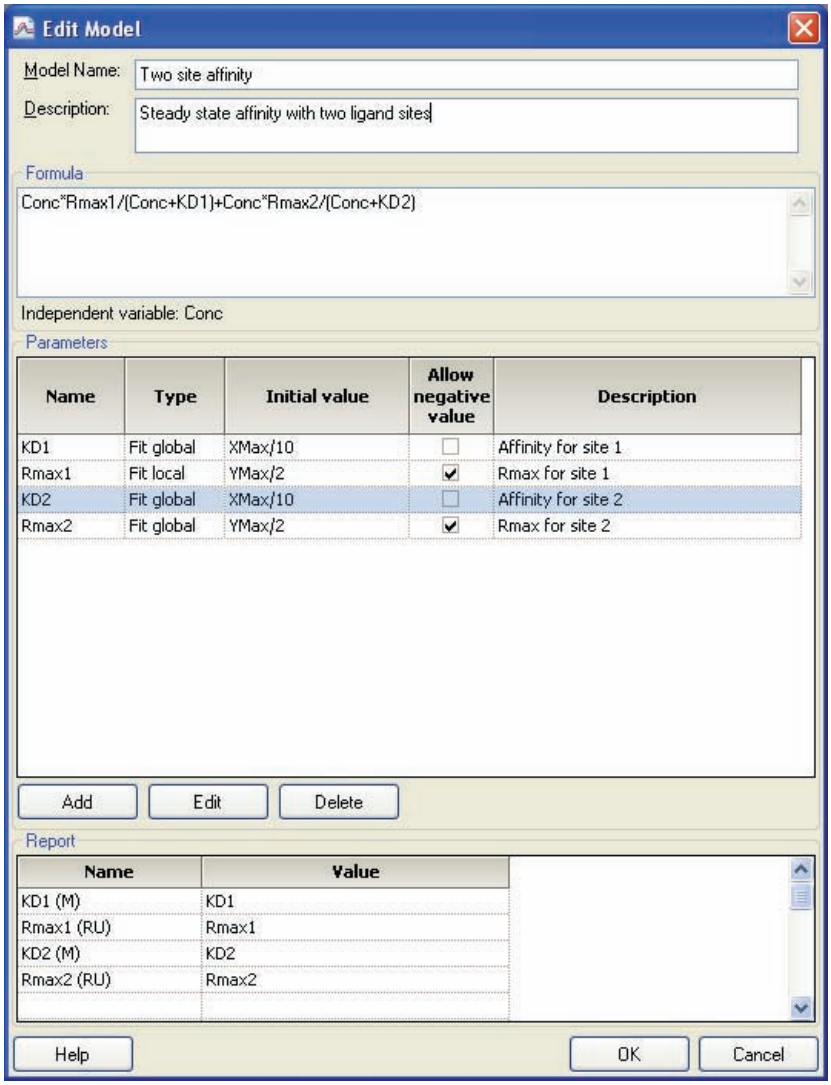
4. Click **Rate Equations** to display the equations generated by the software.




### *D.3.2 Equation models for kinetics and affinity*

Equation models for kinetics are entered as an expression defining response as a function of time. 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.

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




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

# E. System administration

## E.1 Database overview

Biacore X100 saves all data in Oracle Database 10*g* Express Edition. The database is installed on the system controller during the software installation. The database may be accessed via the network from computers with Oracle Client 10*g* Express Edition installed. The Oracle Client is included with the Biacore X100 Software.

The database may be installed on a server and accessed from the system controller via the network. See Section E.7 for more detailed information.

## E.2 Biacore X100 user types

There are two types of users in Biacore X100: users and administrators.

**Users** can view all other users' data, create new sub-folders in their own folder and delete data that they created themselves.

**Administrators** can setup user accounts, change password properties, manage databases (including backup and restoring of databases) and delete any user's data. For more details on what the administrator can do, see the following sections.

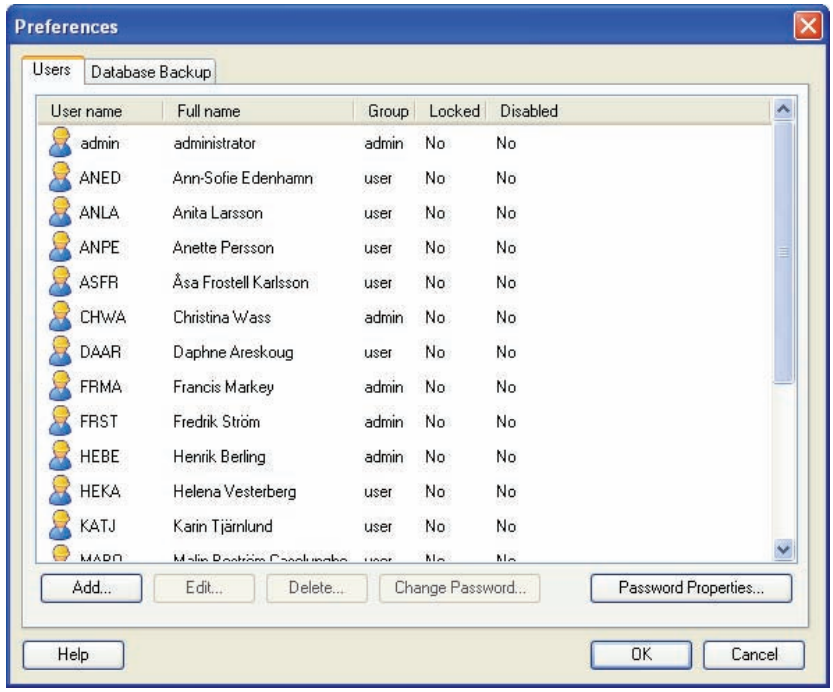
One administrator is set up in the database by default, with username **admin** and password **administrator**.

## E.3 User administration

Only Biacore X100 database administrators can set up new users, edit existing users, manage passwords etc. It is recommended that most of the users belong to the group User and only a few are Biacore X100 administrators.

To access user administration functions, click **Tools:Preferences** in the Biacore X100 Control Software and choose the **Users** tab.

Note: The **Preferences** option is only available in the **Tools** menu when a Biacore X100 administrator is logged in.




## E.4 Database backup

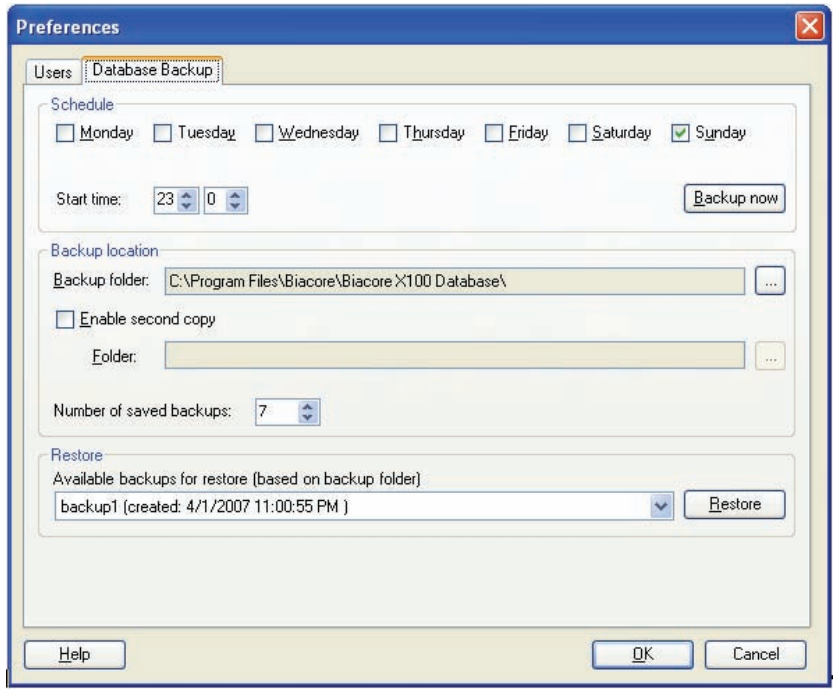
It is important to backup the database at regular intervals. During the installation the backup schedule is set by default to once per week, at 23.00 on Sunday. Only Biacore X100 administrators can change the backup settings. The settings can only be changed on the computer where the database is stored.

The database backup is stored by default in the same directory as the database installation itself. An optional second copy can be enabled for added security: we recommend that you place the second copy on a network drive or a removable drive unit. A typical backup file requires about 2 Gb disk space.

By default up to 7 backups are saved. When the set number of backup is reached, the oldest backup is deleted for each new backup saved.

To view the backup schedule, change it or perform a backup right now, click **Tools:Preferences** in the Biacore X100 Control Software and choose the **Database Backup** tab.

Note: The **Preferences** option is only available in the **Tools** menu when a Biacore X100 administrator is logged in.




## E.5 Restoring a database

You can restore backup data via **Tools:Preferences** in the Control Software, on the **Database Backup** tab. Select which backup to restore and click **Restore**. You cannot select data to restore within a backup.

If your installation has been corrupted so that you cannot access **Tools:Preferences**, first install a new database (see Section E.7) and then restore data to the new empty database.

Note: Restoring a database will overwrite all current database contents including usernames and passwords.

## E.6 Database connections

To view Biacore X100 data you need to have access to the database. If you access the database via the network you will have to define the database connection.

### *E.6.1 Selecting a database at login*

1. In the login dialog, click **Options>>**.


2. Select the appropriate database connection from the scroll-list and click **OK**.

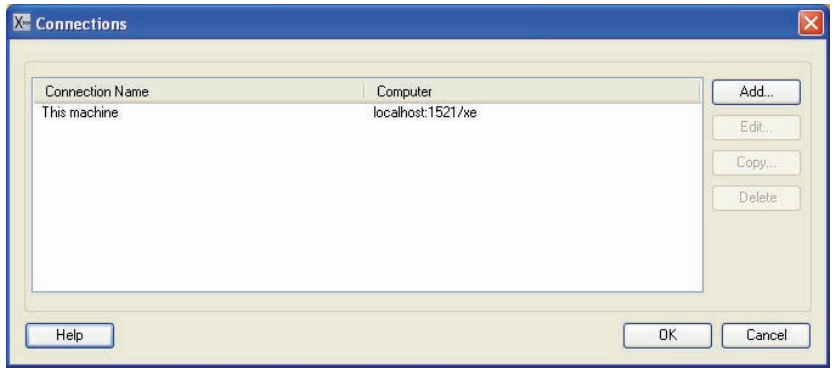
   The default database is **This machine**.

### *E.6.2 Managing databases*

1. In the login dialog on the system controller, click **Options>>.**


2. Click the browse button [...].

3. The Connections dialog opens with several possibilities to manage the database. See the Support Navigator to the right in the software for detailed help.




Details of database setup parameters are given in the installation instructions, available on the Biacore web site. Log in to the download area at www.biacore.com/Downloads to access the instructions.

## E.7 Re-installing the Biacore X100 software

The Biacore X100 database and software are pre-installed on the system controller. In the event that re-installation is required, installation instructions may be found on the Biacore web site at www.biacore.com/Downloads (see above). Instructions are also provided for installing and setting up the database on a network server, and for installing additional copies of the software on other computers that access the database on the system controller.

# Index

HANDBOOK