



Biacore™ X100

Handbook



Table of Contents

1	Introduction	6
2	Safety instructions	7
2.1	Safety precautions	8
2.2	Labels	11
2.3	Emergency stop procedure	12
3	System overview	13
3.1	Biacore X100 system	14
3.2	Biacore terminology	16
3.3	Learning to use Biacore X100	18
3.4	Biacore X100 instrument overview	19
3.5	Biacore X100 software overview	20
	3.5.1 <i>Biacore X100 Software</i>	21
	3.5.2 <i>Getting support</i>	22
4	Biacore X100 basics	23
4.1	Available assays with Biacore X100	24
	4.1.1 <i>Kinetic/Affinity analysis</i>	25
	4.1.2 <i>Binding analysis</i>	26
	4.1.3 <i>Concentration analysis</i>	27
	4.1.4 <i>Solvent correction</i>	28
4.2	Interacting molecules in the assay	29
	4.2.1 <i>Attachment methods</i>	30
	4.2.2 <i>Overview of sensor chips</i>	31
	4.2.3 <i>Tips on how to select which molecule to attach</i>	32
4.3	Buffer recommendations	33
4.4	Assay duration	34
5	Operation	35
5.1	Starting the instrument	36
	5.1.1 <i>Starting the instrument from shutdown</i>	37
	5.1.2 <i>Starting the instrument from standby</i>	41
5.2	Preparing and loading buffer	42
5.3	Inserting, removing or changing the sensor chip	43
	5.3.1 <i>Sensor chip and detection</i>	44
	5.3.2 <i>Inserting a sensor chip</i>	45
5.4	Analysis temperature	47
5.5	Preparing and loading samples and reagents	48
	5.5.1 <i>Sample compartment</i>	49
	5.5.2 <i>Samples and reagents</i>	50
	5.5.3 <i>Loading the rack</i>	51
5.6	Starting a run	52
5.7	After a run	53
	5.7.1 <i>Chip handling after a run</i>	54
	5.7.2 <i>Standby or shutdown</i>	55

6	Developing and running assays	56
6.1	Biacore X100 Control Software	57
6.1.1	<i>Logging in to Biacore X100 Control Software</i>	58
6.1.2	<i>Start view</i>	59
6.1.3	<i>Support Navigator</i>	60
6.1.4	<i>Database contents and display</i>	61
6.2	Creating assay workflows	64
6.2.1	<i>What is an assay workflow?</i>	65
6.2.2	<i>Overview of steps in a workflow</i>	66
6.2.3	<i>Creating a workflow</i>	68
6.2.4	<i>Editing a workflow</i>	70
6.2.5	<i>Automatic display of result data</i>	71
6.3	Other ways to run assays on Biacore X100	72
6.3.1	<i>Using wizards</i>	73
6.3.2	<i>Manual runs</i>	75
7	Evaluation	76
7.1	Starting the Evaluation Software	77
7.2	Evaluation Software – general features	78
7.2.1	<i>Start view</i>	79
7.2.2	<i>Opening items</i>	80
7.2.3	<i>Combining results from multiple runs</i>	81
7.2.4	<i>The Evaluation explorer</i>	82
7.2.5	<i>Adding new evaluation items</i>	83
7.2.6	<i>Right-click menus in the work area</i>	84
7.3	Presenting data	85
7.3.1	<i>Sensorgram windows</i>	86
7.3.2	<i>Plot windows</i>	87
7.3.3	<i>Bar charts</i>	88
7.3.4	<i>Kinetics/Affinity analysis</i>	89
7.3.5	<i>Concentration analysis</i>	91
7.3.6	<i>Solvent correction</i>	93
7.3.7	<i>Other evaluation functions</i>	94
7.4	Workflow for assay evaluation	95
8	Biacore X100 Plus Package	96
8.1	In-line degasser	97
8.2	Variable analysis temperature control	98
8.3	Custom assay wizard	99
8.4	Concentration analysis wizards	101
8.4.1	<i>Using calibration</i>	102
8.4.2	<i>Calibration-free-concentration analysis (CFCA)</i>	103
8.5	Solvent correction	104
8.6	Custom immobilization	105
8.7	User-defined fitting models for kinetics and affinity	106
9	Maintenance	107
9.1	User maintenance operations	108
9.2	Standby	111
9.3	Shutdown	112

10 Troubleshooting	114
10.1 Instrument related troubleshooting	115
10.2 Assay related troubleshooting	119
Appendix A: Installation requirements	120
A.1 General guidelines	121
A.2 Space requirements	122
A.3 Mains power supply	123
A.4 Heating and ventilation	124
A.5 Moving the instrument within the lab	125
A.5.1 <i>Shutting down the system</i>	126
A.5.2 <i>Connecting to mains power</i>	127
A.5.3 <i>Connecting the instrument to the PC</i>	128
Appendix B: Specifications	129
B.1 General	130
B.2 Instrument	131
B.3 System controller and software	132
B.4 Sample rack	133
B.5 Liquid containers provided	134
B.6 Chemical resistance	135
Appendix C: Technical description	136
C.1 Detection principle	137
C.1.1 <i>Surface plasmon resonance</i>	138
C.1.2 <i>What SPR measures</i>	140
C.2 Detection system	141
C.2.1 <i>Sensor chip</i>	142
C.2.2 <i>Integrated μ-Fluidic Cartridge (IFC)</i>	143
C.2.3 <i>Flow cells</i>	144
C.3 Pump compartment	145
Appendix D: Fitting equations and models	146
D.1 Curve fitting principles	147
D.1.1 <i>Fitting procedure</i>	148
D.1.2 <i>Local and global parameters</i>	149
D.2 Statistical parameters	150
D.2.1 <i>Report points</i>	151
D.2.2 <i>Significance of fitted parameters</i>	152
D.3 Predefined models - kinetics and affinity	153
D.3.1 <i>Mass transfer parameters</i>	154
D.3.2 <i>Kinetics – 1:1 binding</i>	155
D.3.3 <i>Kinetics – Bivalent Analyte</i>	156
D.3.4 <i>Kinetics – Heterogeneous Analyte</i>	157
D.3.5 <i>Kinetics – Heterogeneous Ligand</i>	158
D.3.6 <i>Kinetics – Two State Reaction</i>	159
D.3.7 <i>Affinity – Steady State 1:1</i>	160
D.4 Creating and editing models	161
D.4.1 <i>Interaction models for kinetics</i>	162
D.4.2 <i>Equation models for kinetics and affinity</i>	166

D.5	Predefined models - concentration analysis	168
D.5.1	<i>Using calibration</i>	169
D.5.2	<i>Calibration-free concentration analysis (CFCA)</i>	170
Appendix E: Solvent correction		172
E.1	Background	173
E.2	How solvent correction works	174
E.3	When should solvent correction be used?	175
E.4	Applying solvent correction	176
Appendix F: System administration		178
F.1	Database overview	179
F.2	Biacore X100 user types	180
F.3	User administration	181
F.4	Database backup	182
F.5	Restoring a database	183
F.6	Database connections	184
F.6.1	<i>Selecting a database at login</i>	185
F.6.2	<i>Managing databases</i>	186
F.7	Re-installing the Biacore X100 software	188
Index		189

1 Introduction

This section contains important user information required before using the instrument.

Biacore™ X100 is intended for research use only and should not be used for diagnostic purposes in any clinical or *in vitro* procedures.

This edition of the Biacore X100 Handbook applies to systems running software v2.1.

Read this before operating the product



All users must read the entire *Operating Instructions* before installing, operating, or maintaining the product.

Always keep the *Operating Instructions* at hand when operating the product.

Do not install, operate, or perform maintenance on the product in any other way than described in the user documentation. If you do, you may be exposed or expose others to hazards that can lead to personal injury and you may cause damage to the equipment.

Notes and tips

Note: *A note is used to indicate information that is important for trouble-free and optimal use of the product.*

Tip: *A tip contains useful information that can improve or optimize your procedures.*

Typographical conventions

Software items are identified in the text by ***bold italic*** text.

Hardware items are identified in the text by **bold** text.

Tip: *The text can include clickable hyperlinks to reference information.*

2 Safety instructions

This chapter describes safety precautions, labels and symbols that are attached to the equipment. In addition, the chapter describes emergency and recovery procedures.

In this chapter

Section	See page
2.1 Safety precautions	8
2.2 Labels	11
2.3 Emergency stop procedure	12

Important



WARNING

All users must read and understand the entire contents of this general safety chapter, and the specific safety precautions information in each subsequent chapter of this manual to become aware of the hazards involved.

2.1 Safety precautions

Introduction

Biacore X100 is powered by mains voltage and handles materials that can be hazardous.

All users must read the entire *Biacore X100 Operating Instructions (28961142)* before installing, operating, or maintaining the product.

Definitions

This user documentation contains safety notices (WARNING, CAUTION, and NOTICE) concerning the safe use of the product. See definitions below.



WARNING

WARNING indicates a hazardous situation which, if not avoided, could result in death or serious injury. It is important not to proceed until all stated conditions are met and clearly understood.



CAUTION

CAUTION indicates a hazardous situation which, if not avoided, could result in minor or moderate injury. It is important not to proceed until all stated conditions are met and clearly understood.



NOTICE

NOTICE indicates instructions that must be followed to avoid damage to the product or other equipment.

Safety information

Biacore X100 Instrument contains mains voltage and handles liquids that may be hazardous.

Safety instructions



WARNING

Biacore X100 should only be operated by properly qualified personnel. Read this manual before operating the instrument.

**WARNING**

The instrument must be connected to a grounded mains socket.

**WARNING**

Do not block the rear or side panels of the instrument. The power switch must always be easy to access. The power cord must always be easy to disconnect.

**WARNING**

Always wear appropriate protective clothing and glasses during operation and maintenance of Biacore X100. Use required safety equipment when handling hazardous substances.

**WARNING**

A fume hood or similar ventilation system shall be installed when flammable or noxious substances are used.

**WARNING**

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**

Decontaminate the equipment before decommissioning to ensure that hazardous residues are removed.

**CAUTION**

Waste tubes and containers shall be secured and sealed to prevent accidental spillage.

**CAUTION**

Make sure the waste container is dimensioned for maximum possible volume when the instrument is left unattended



CAUTION

Do not touch the pumps while they are moving.



CAUTION

Biacore X100 weighs 47 kg. At least two people are required to lift the instrument.


2.2 Labels

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.

Symbol	Meaning
	Warning! Read the user documentation before using the system. Do not open any covers or replace parts unless specifically stated in the user documentation.

2.3 Emergency stop procedure

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

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



NOTICE

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.
---	--



NOTICE

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

Step	Action
1	Turn on mains power if it is switched off and check that the instrument starts normally (see Section 5.1 Starting the instrument, on page 36).
2	If you need to clean the liquid handling system, eject the sensor chip and insert a maintenance chip. See Chapter 9 Maintenance, on page 107 for further instructions.

3 System overview

This chapter gives an overview of Biacore X100, and a brief description of its function and the Biacore X100 software.

In this chapter

Section		See page
3.1	Biacore X100 system	14
3.2	Biacore terminology	16
3.3	Learning to use Biacore X100	18
3.4	Biacore X100 instrument overview	19
3.5	Biacore X100 software overview	20

3.1 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 3.1: The Biacore X100 instrument.

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 a change in refractive index at the sensor surface that is proportional to the change in mass concentration. The change is detected in real time and data is presented in a sensorgram (SPR response plotted against time, see the Figure below). 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.

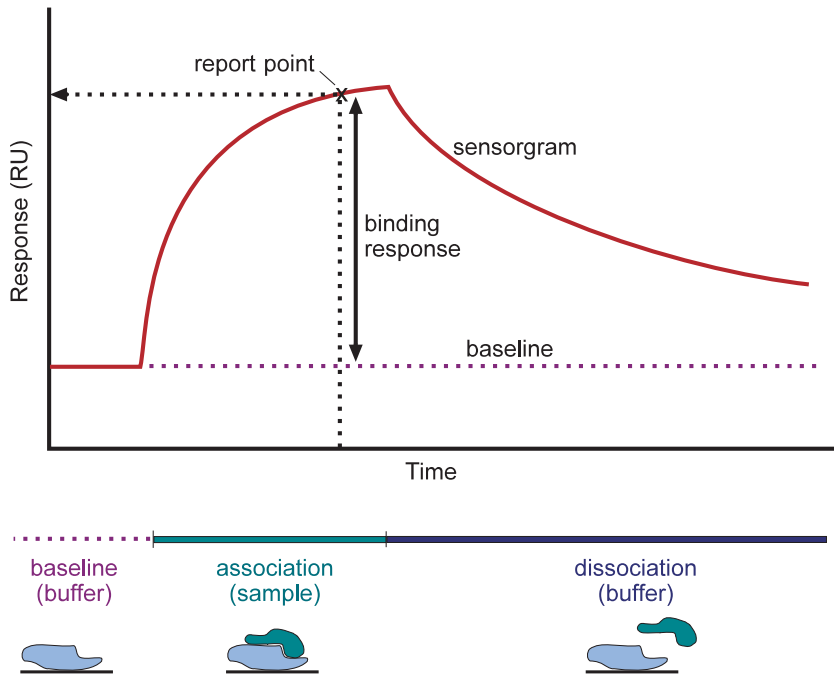


Figure 3.2: Sensorgram and interaction.

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 (see [Fig. 3.4, on page 17](#)).

3.2 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* (see the Figure below). (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 (see the Figure below).

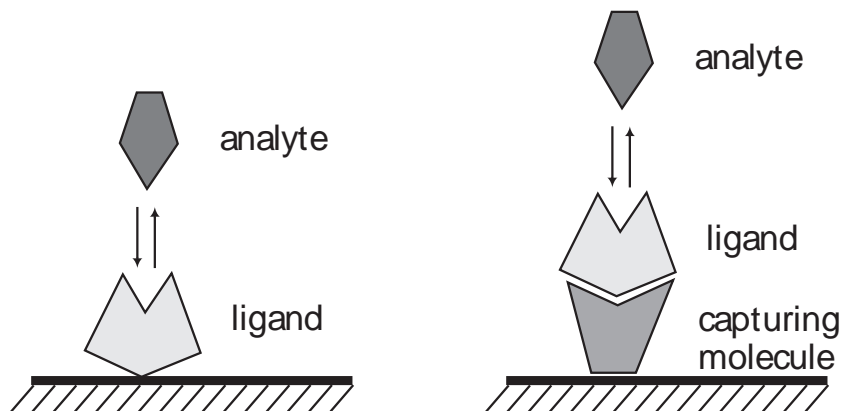


Figure 3.3: The ligand is the interaction partner that is attached to the sensor surface. Ligand may be immobilized directly on the surface (left) or attached through binding to an immobilized capturing molecule (right). The analyte is free in solution and binds to the immobilized ligand.

- 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 (see the Figure below). 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 (see the Figure below).

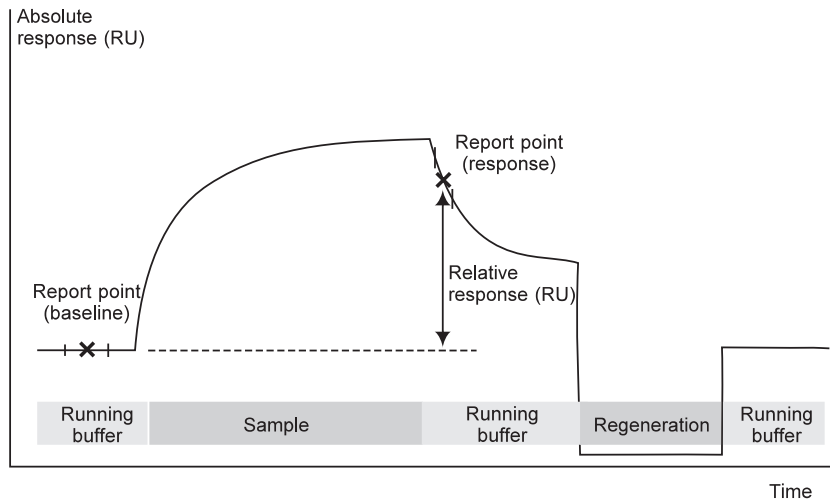


Figure 3.4: Schematic illustration of a sensorgram. The bars below the sensorgram curve indicate the solutions that pass over the sensor surface.

3.3 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 Cytiva web site
- performing a guided assay in the Getting Started Tutorial using a Getting Started Kit (kit ordered separately)

3.4 Biacore X100 instrument overview

The Figure below illustrates the main components of Biacore X100 instrument.



Figure 3.5: Biacore X100 instrument components.

Part	Function
1	Buffer tray
2	Buffer bottle
3	Two buffer tubes
4	Lower front door with pump compartment behind, see also Section 5.1 Starting the instrument, on page 36
5	Status indicators, see also Appendix C Technical description, on page 136
6	Upper front door with sensor chip port behind
7	Sample compartment, see also Section 5.5.1 Sample compartment, on page 49
8	Waste tubing
9	Waste bottle
10	Waste tray

3.5 Biacore X100 software overview

Biacore X100 software is designed to make the system easy and efficient for both new and experienced users. All assay setup and result information is conveniently collected and stored in the Biacore X100 database.

In this section

Section	See page
3.5.1 Biacore X100 Software	21
3.5.2 Getting support	22

3.5.1 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 6 Developing and running assays, on page 56](#). For more information on evaluation, see [Chapter 7 Evaluation, on page 76](#).

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 support for solvent correction for working with low molecular weight analytes in organic solvents. For more information on Biacore X100 Plus Package, see [Chapter 8 Biacore X100 Plus Package, on page 96](#).

3.5.2 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 Cytiva'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 X100 pages on cytiva.com/biacore (you must register on the web-site using the product key for your system as authorization for web-based access).

4 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 [cytiva.com/biacore](https://www.cytiva.com/biacore).

In this chapter

Section		See page
4.1	Available assays with Biacore X100	24
4.2	Interacting molecules in the assay	29
4.3	Buffer recommendations	33
4.4	Assay duration	34

4.1 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.

In this section

Section	See page
4.1.1 Kinetic/Affinity analysis	25
4.1.2 Binding analysis	26
4.1.3 Concentration analysis	27
4.1.4 Solvent correction	28

4.1.1 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.

Biacore X100 supports kinetics and affinity analysis using both single- and multi-cycle formats, by examining the sensorgrams obtained over a range of sample concentrations injected over the same surface.

- In single-cycle analyses, the analyte is injected with increasing concentrations in a single cycle. The surface is not regenerated between injections.
- In multi-cycle analyses, each sample concentration is run in a separate cycle. Analyte is removed by dissociation or regeneration at the end of each cycle.

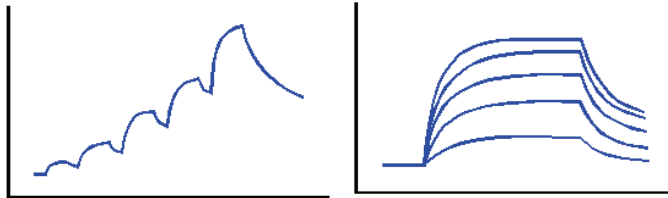


Figure 4.1: Comparison of sensorgrams from single-cycle kinetic assay (left) and multicycle kinetic assay (right).

The multi-cycle approach is well established but needs efficient regeneration between cycles. Single-cycle analysis is a novel and convenient approach, and is valuable in cases where it is difficult to find adequate regeneration conditions.

Both approaches are evaluated with the same software tools and give equivalent results.

4.1.2 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.

4.1.3 Concentration analysis

Measurement of analyte concentration in samples is only available if you have the Biacore X100 Plus Package installed. Two approaches are supported:

- *Using calibration*, where known samples are measured to create a calibration curve, and concentrations in unknown samples are determined by reference to the calibration curve.
- *Calibration-free concentration analysis (CFCA)*, where analyte concentration is determined without reference to a calibration curve. The concentration is calculated from the diffusion properties of the analyte molecule and the binding rate of analyte to the surface under diffusion-limited conditions.

The principles of concentration measurement with Biacore are discussed in detail in the *Biacore Concentration Analysis Handbook*.

4.1.4 Solvent correction

Interaction analysis with low molecular weight compounds that require organic solvents (commonly dimethyl sulfoxide, DMSO) to maintain solubility is complicated by the high bulk responses caused by DMSO in comparison to the intrinsically low responses obtained from small molecules. Solvent correction is a procedure supported in the optional Biacore X100 Plus Package that corrects observed responses for the effects of DMSO and improves the robustness of measurements with small molecules.

4.2 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 both larger structures such as cells and viruses and low molecular weight compounds.

In this section

Section	See page
4.2.1 Attachment methods	30
4.2.2 Overview of sensor chips	31
4.2.3 Tips on how to select which molecule to attach	32

4.2.1 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 4.2.3](#) *Tips on how to select which molecule to attach, on page 32.*

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.

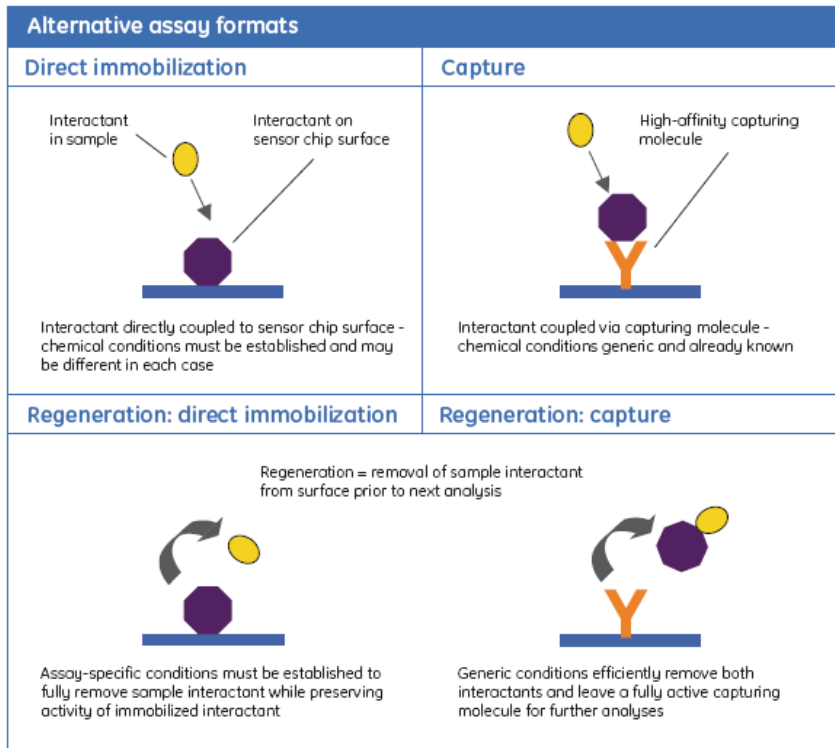


Figure 4.2: Alternative assay formats.

4.2.2 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 cytiva.com/biacore.

4.2.3 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 Cytiva 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. With the Custom Assay Wizard in the Biacore X100 Plus Package, you can vary both interactants (capturing a different ligand in different cycles as well as injecting different samples).
- 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 may be less suitable as analytes since they 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.

4.3 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 HBS-EP+ buffer from Cytiva. 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 nonspecific binding of material to the sensor surface.

Buffers for sample preparation

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

4.4 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.

5 Operation

This chapter describes basic operation of Biacore X100 instrument. Install the instrument and the PC as described in [Appendix A Installation requirements, on page 120](#). For information on how to select assays, optimize assay conditions and perform a run, see [Chapter 6 Developing and running assays, on page 56](#).

In this chapter

Section		See page
5.1	Starting the instrument	36
5.2	Preparing and loading buffer	42
5.3	Inserting, removing or changing the sensor chip	43
5.4	Analysis temperature	47
5.5	Preparing and loading samples and reagents	48
5.6	Starting a run	52
5.7	After a run	53

5.1 Starting the instrument

If the instrument is shut down, start according to [Section 5.1.1 Starting the instrument from shutdown, on page 37](#).

If the instrument is in standby mode, start according to [Section 5.1.2 Starting the instrument from standby, on page 41](#).

In this section

Section	See page
5.1.1 Starting the instrument from shutdown	37
5.1.2 Starting the instrument from standby	41

5.1.1 Starting the instrument from shutdown

**NOTICE**

If you have the Biacore X100 Plus Package, always keep the buffer tubes in liquid when the instrument is powered on to prolong the life of the degasser.

Step	Action
1	Switch on the PC.

Step	Action
------	--------

2	Switch on the instrument at the rear.
---	---------------------------------------

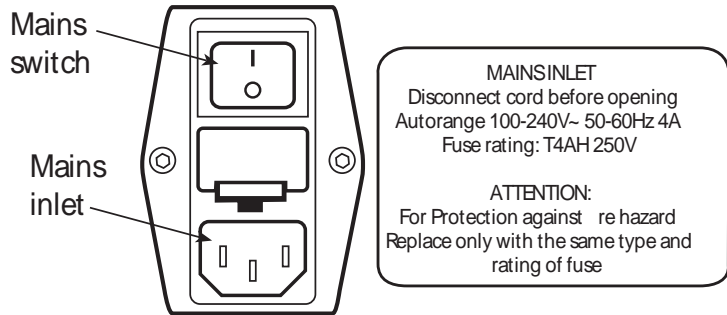


Figure 5.1: Mains input panel at the rear of the instrument.

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.

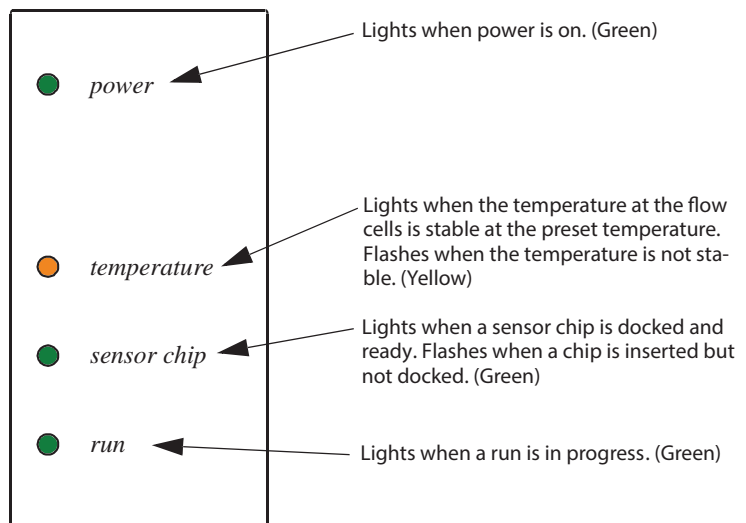


Figure 5.2: Status indicators on the front panel.

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

Step	Action
------	--------

- | | |
|---|--|
| 4 | <p>In the login-dialog, enter your user name and password and verify that the correct database is selected in the Database connection list. Click Log in. Your Windows username and password (case-sensitive) are valid as credentials for Biacore X100 Control Software. If only the username is entered, the current domain for the user logged in at the computer is used. To change domain, enter your domain\username in the field.</p> |
|---|--|

Note:

Different Windows accounts can be used for logging in to the system controller and Biacore X100 Control Software.

- | | |
|---|---|
| 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 Buffer preparation, on page 42 . |
| 7 | Ensure that the waste bottle on the right-hand tray is empty and the waste tube is inserted in the bottle. |
| 8 | <p>The system event log is displayed in the software and the startup procedure is recommended. Click Run now and follow the instructions on the screen.</p> <p>A previously used chip can be docked for the startup procedure (see Section 5.3 Inserting, removing or changing the sensor chip, on page 43 for instructions on docking a sensor chip).</p> |

Step	Action
------	--------

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



Figure 5.3: Closing the cover of the peristaltic pump.

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

5.1.2 Starting the instrument from standby

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

Step	Action
------	--------

- | | |
|---|--|
| 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 verify that the correct database is selected in the Database connection list. Click Log in . Your Windows username and password (case-sensitive) are valid as credentials for Biacore X100 Control Software. If only the username is entered, the current domain for the user logged in at the computer is used. To change domain, enter your domain\username in the field. |

Note:

Different Windows accounts can be used for logging into the system controller and Biacore X100 Control Software.

- | | |
|---|---|
| 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 Buffer preparation, on page 42 . |
| 5 | Ensure that the waste bottle on the right-hand tray is empty and the waste tube is inserted in the bottle. |

5.2 Preparing and loading buffer

Buffer preparation

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

Use degassed deionized water when preparing buffers 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 a freshly prepared buffer filtered through a 0.22 µm filter to remove particles. A volume of 200 ml is suitable for use during 24 hours.

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 5.1 Starting the instrument, on page 36](#) and [Section 5.3 Inserting, removing or changing the sensor chip, on page 43](#)).

If the system is in standby, follow the instructions in [Changing buffer, on page 42](#).

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.

5.3 Inserting, removing or changing the sensor chip

**NOTICE**

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.

In this section

Section	See page
5.3.1 Sensor chip and detection	44
5.3.2 Inserting a sensor chip	45

5.3.1 Sensor chip and detection

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



Sensor chip types

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

There are several different types of sensor chips appropriate for different molecules with different attachment chemistries. A brief overview of sensor chip types is given in [Section 4.2.2 Overview of sensor chips, on page 31](#).

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 C Technical description, on page 136](#) for a description of the SPR principle and more detailed information on the detection system.

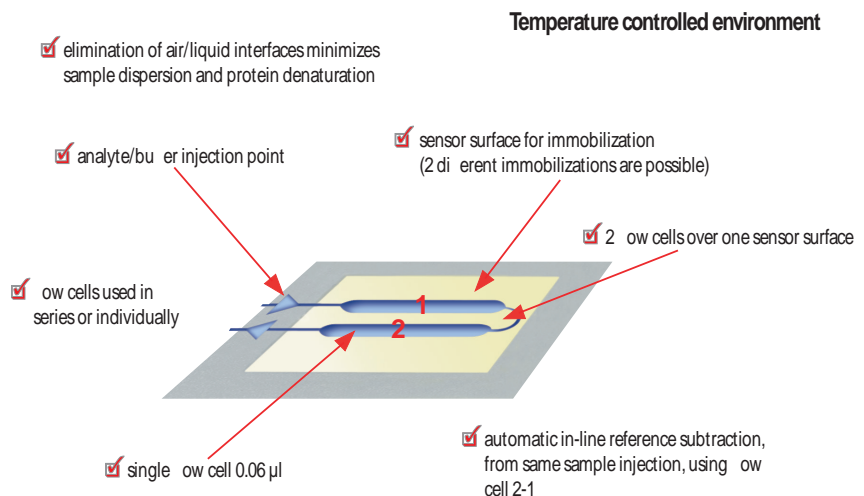


Figure 5.4: Flow cells on the sensor chip.

5.3.2 Inserting a sensor chip

Follow the steps below to insert a sensor chip into the instrument.

- | Step | Action |
|------|--|
| 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. |
|---|--------------------------|



- | | |
|---|-----------------------------------|
| 4 | If required, remove the old chip. |
|---|-----------------------------------|



Step **Action**

- 5 Insert the chip with the text on the upper side and the arrow pointing into the instrument.



- 6 Push the chip slide all the way in.



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

5.4 Analysis temperature

The SPR signal 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 8.2 Variable analysis temperature control, on page 98](#)).

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.

Online - COM1	Current temp: 24.89 °C	Sensor chip: CM5
		Running standby, remaining time: 4,0 days
Online - COM1	Set temperature: 25 °C	Sensor chip: CM5
		Running standby, remaining time: 4,0 days

5.5 Preparing and loading samples and reagents

In this section

Section		See page
5.5.1	Sample compartment	49
5.5.2	Samples and reagents	50
5.5.3	Loading the rack	51

5.5.1 Sample compartment

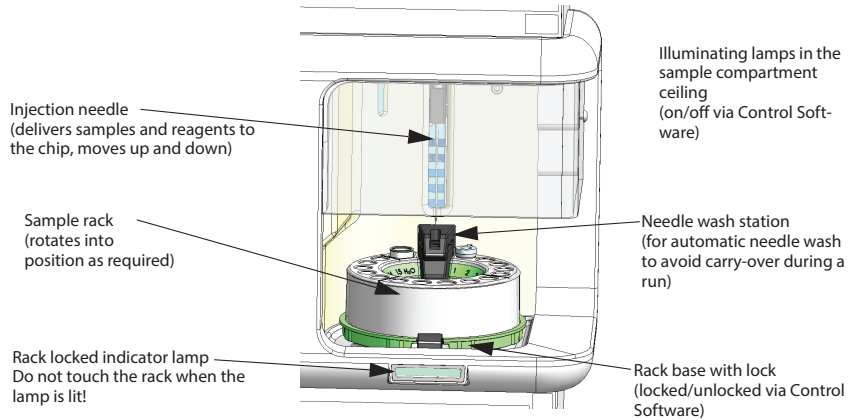


Figure 5.5: The sample compartment.



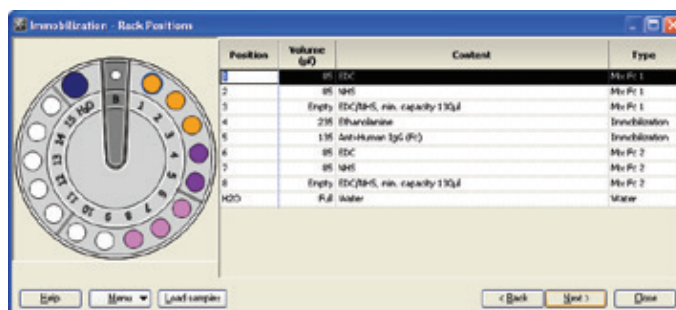
NOTICE

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 damaged if the rack is prevented from moving.

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

5.5.2 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 when the assay is set up. 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.

Step Action

- 1 Prepare the samples and reagents in 1.5 ml conical vials (Cytiva) according to the volumes and concentrations in the rack position list and cap them to avoid evaporation during the run. Make sure there are no visible air bubbles in the tubes. Use only caps supplied for this purpose by Cytiva.
- 2 Fill the 4 ml vial with distilled 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 cleaning the needle during the run and during standby.
- 3 Load the rack as described in [Section 5.5.3 Loading the rack, on page 51](#).

5.5.3 Loading the rack

Step	Action
1	<p>Click the Load Samples button in the Rack Positions dialog. You can also load the rack using the Load Samples icon in the toolbar if you do not have an application wizard open.</p>  The icon shows a circular rack with several colored spots (red, blue, green) and a black arrow pointing downwards from the center.
2	<p>Wait until the Rack locked indicator below the rack compartment is switched off.</p>
3	<p>Lift out the rack and place the sample and reagent vials and the water vial according to the rack position list.</p> <p>Note: <i>Load samples in the rack outside the instrument.</i></p>
4	<p>Insert the rack in the sample compartment and make sure it sits properly on the rack base.</p>
5	<p>Click OK in the Load Samples dialog.</p>

5.6 Starting a run

Runs are started from a workflow, via a wizard or via a manual run. See [Chapter 6 Developing and running assays, on page 56](#) for more information.

Before starting a run, check

- that there is sufficient buffer in the buffer bottle, and that both buffer tubings are properly inserted in the buffer
- that the waste bottle has sufficient space for the waste from the run and that the waste tubing is properly inserted in the bottle cap
- that samples and reagents are in position in the sample rack, in properly capped vials
- that an uncapped vial with water is in the rack position marked **H₂O**
- that the rack is properly mounted and locked (the Rack locked indicator should be lit).

5.7 After a run

After a run, take care of the chip (see [Section 5.7.1 Chip handling after a run, on page 54](#)) and choose whether to leave the instrument in standby or perform a shutdown (see [Section 5.7.2 Standby or shutdown, on page 55](#)).

In this section

Section	See page
5.7.1 Chip handling after a run	54
5.7.2 Standby or shutdown	55

5.7.1 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. 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.

5.7.2 Standby or shutdown

If the instrument will be used next...	Action
...within 7 days	Prepare the system for Standby, see Section 9.2 Standby, on page 111 .
...after more than 7 days	Perform a Shutdown, see Section 9.3 Shutdown, on page 112 .

6 Developing and running assays

In this chapter

Section		See page
6.1	Biacore X100 Control Software	57
6.2	Creating assay workflows	64
6.3	Other ways to run assays on Biacore X100	72

6.1 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 use solvent correction procedures to improve performance with low molecular weight analytes in organic solvents.

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

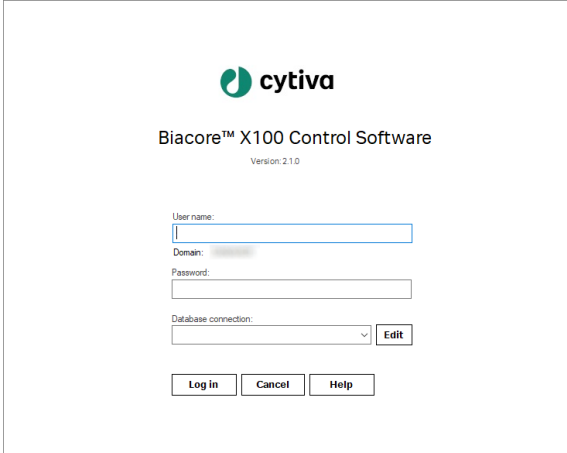
In this section

Section	See page
6.1.1 Logging in to Biacore X100 Control Software	58
6.1.2 Start view	59
6.1.3 Support Navigator	60
6.1.4 Database contents and display	61

6.1.1 Logging in to Biacore X100 Control Software

Step	Action
------	--------

- | | |
|---|--|
| 1 | Start Biacore X100 Control Software from the Windows start menu. The start menu shortcut is installed in the Biacore group. |
|---|--|



- | | |
|---|--|
| 2 | Enter your user name and password. Your Windows user name and password are valid as credentials for Biacore X100 Control Software. |
|---|--|

Note:

Account credentials do not have to be the same for login to Windows and the Biacore X100 Control Software. Make sure that valid credentials are used in both cases.

Verify that the correct database is selected in the **Database connection** list.

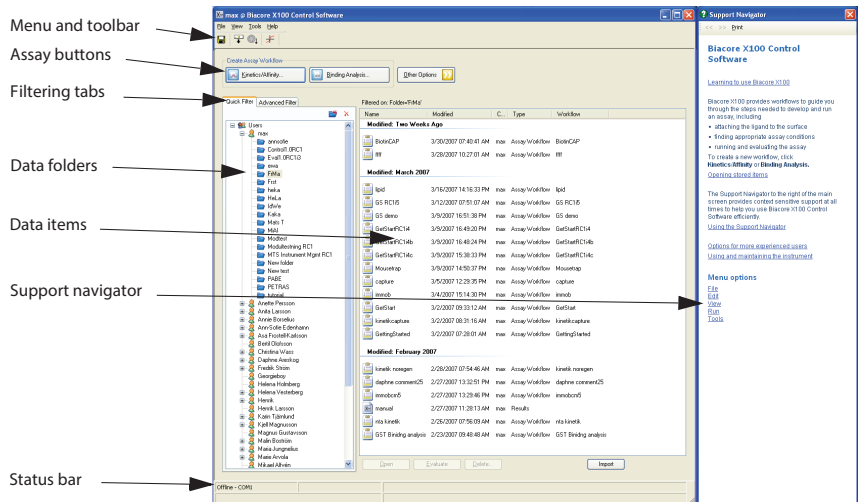
- | | |
|---|-----------------------|
| 3 | Click Log in . |
|---|-----------------------|

Your Biacore X100 administrator can provide you with the database information. For detailed information on database, see [Appendix F System administration, on page 178](#).

6.1.2 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 6.2 Creating assay workflows, on page 64](#).

- There are two **Filter tabs**:

The **Quick Filter** tab displays all users in the database, their different subfolders in the database and allows for filtering of data via users.

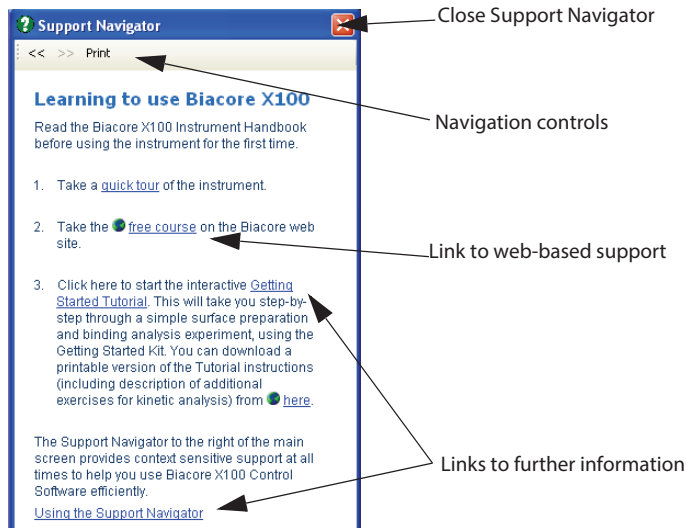
The **Advanced Filter** tab allows for more advanced filtering.

For details, see [Section 6.1.4 Database contents and display, on page 61](#) 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 current step in the software. As you move through the software the Support Navigator contents are continuously updated. For details, see [Section 6.1.3 Support Navigator, on page 60](#).

6.1.3 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 Cytiva web site, after registration on the site with your Biacore X100 product key.



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

Retrace your steps using the navigation controls.





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

Note: *Links from the Support Navigator to the web-based support are marked with the symbol . A connection to the internet is required to follow these links.*

6.1.4 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 users with the role **BiacoreX100Administrators** and the user who created the data 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. 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. Results from assay development steps are evaluated in the Control Software. 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

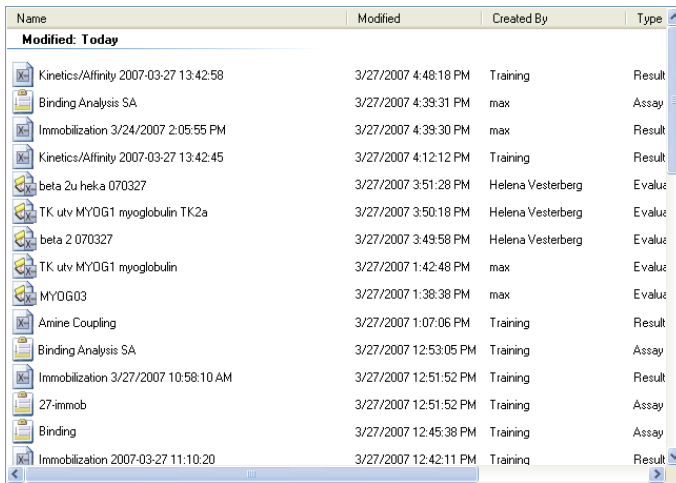
The start view displays the items currently filtered from the database. The filtering selection is made on the **Quick Filter** or **Advanced Filter** tabs.

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

6 Developing and running assays


6.1 Biacore X100 Control Software

6.1.4 Database contents and display



Name	Modified	Created By	Type
Modified: Today			
Kinetics/Affinity 2007-03-27 13:42:58	3/27/2007 4:48:18 PM	Training	Result
Binding Analysis SA	3/27/2007 4:39:31 PM	max	Assay
Immobilization 3/24/2007 2:05:55 PM	3/27/2007 4:39:30 PM	max	Result
Kinetics/Affinity 2007-03-27 13:42:45	3/27/2007 4:12:12 PM	Training	Result
beta 2u heka 070327	3/27/2007 3:51:28 PM	Helena Vesterberg	Evaluat
TK utv MYOG1 myoglobin TK2a	3/27/2007 3:50:18 PM	Helena Vesterberg	Evaluat
beta 2 070327	3/27/2007 3:49:58 PM	Helena Vesterberg	Evaluat
TK utv MYOG1 myoglobin	3/27/2007 1:42:48 PM	max	Evaluat
MYOG03	3/27/2007 1:38:38 PM	max	Evaluat
Amine Coupling	3/27/2007 1:07:06 PM	Training	Result
Binding Analysis SA	3/27/2007 12:53:05 PM	Training	Assay
Immobilization 3/27/2007 10:58:10 AM	3/27/2007 12:51:52 PM	Training	Result
27-immob	3/27/2007 12:51:52 PM	Training	Assay
Binding	3/27/2007 12:45:38 PM	Training	Assay
Immobilization 2007-03-27 11:10:20	3/27/2007 12:42:11 PM	Training	Result

Figure 6.1: 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 subfolder. You can only create sub-folders in your own main folder.

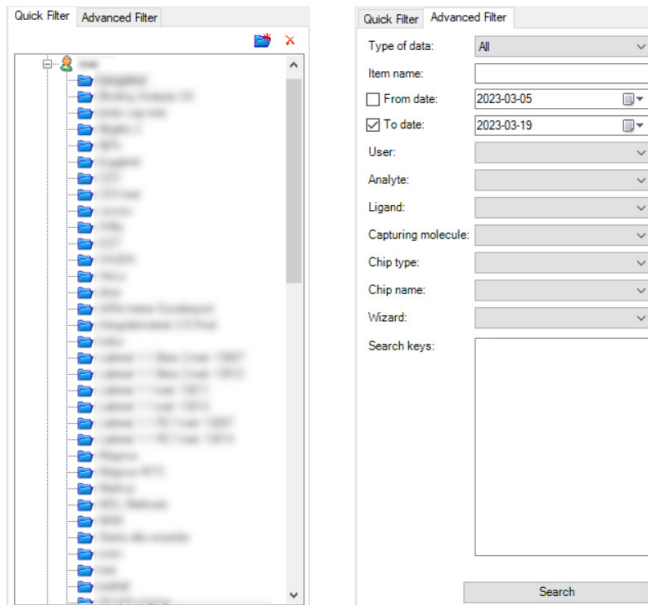


Figure 6.2: All users displayed for a specific Biacore X100 database to the left. The Advanced filtering setting to the right will display all items created March 19, 2023 or earlier.

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.
- click the **Evaluate** button to open the item in the Evaluation Software (only 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 Biacore X100 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.

6.2 Creating assay workflows

In this section

Section		See page
6.2.1	What is an assay workflow?	65
6.2.2	Overview of steps in a workflow	66
6.2.3	Creating a workflow	68
6.2.4	Editing a workflow	70
6.2.5	Automatic display of result data	71

6.2.1 What is an assay 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 one run. For some assay formats, sensor surface preparation is performed as part of the assay step. Assay development steps are available to help you find the best conditions.

Workflows give you recommendations for choice of sensor surface, ligand attachment methods and steps in assay development and execution according to the properties of your interactants. The recommendations use reagent kits from Cytiva 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 provided as defaults in assay development and assay steps. Some settings may be fixed for certain assay types and sensor chip choices. 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 6.3 Other ways to run assays on Biacore X100, on page 72](#)).

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.

6.2.2 Overview of steps in a workflow

A workflow will contain some or all of the steps listed below (see [Fig. 6.4, on page 69](#)). 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 and enter the settings manually in the workflow. Settings that are entered in a workflow are provided as defaults in the subsequent workflow steps. If no settings are entered in the workflow, default values included in the software will be used.

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 Cytiva 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 (see the *Biacore Sensor Surface Handbook* for more information).

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 CAP, Sensor Chip NTA, Sensor Chip L1).

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 Cytiva 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.

6.2.3 Creating a workflow

For detailed support see the Support Navigator in the software.

Step	Action
------	--------

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



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

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

A screenshot of a software dialog box titled 'Create Assay Workflow - Kinetics/Affinity'. The dialog has a blue header bar. Below the header, there is a section titled 'Choose ligand attachment approach'. Under this section, there is a sub-section 'Ligand details'. The 'Ligand name:' field contains the text 'anti-b2micro'. Below this, there are two dropdown menus. The first is labeled 'My ligand is...' and has the selected option '...an antibody'. The second is labeled 'My antibody is...' and has the selected option '...a mouse antibody'.

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

Step Action

- 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.

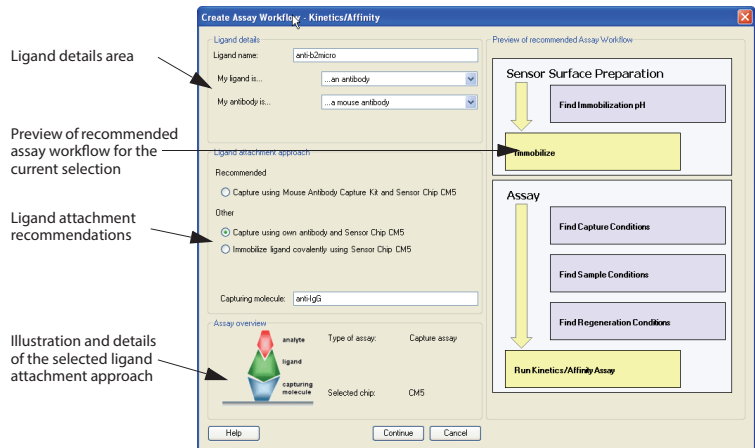


Figure 6.3: Example of the creation of a Kinetics/Affinity workflow with a preview of the recommended assay workflow to the right in the dialog.

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

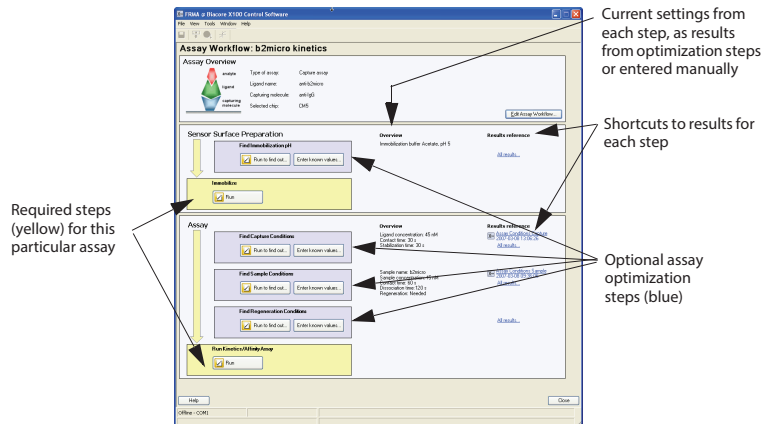


Figure 6.4: Example of a saved Kinetics/Affinity workflow with required steps in yellow boxes and optional assay development steps in blue boxes. Runs are started from the buttons in the boxes.

- 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.

6.2.4 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.

6.2.5 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.

6.3 Other ways to run assays on Biacore X100

Apart from workflow assays you can also run assays using the wizards outside a workflow or using manual runs. See the following sections for more details.

In this section

Section	See page
6.3.1 Using wizards	73
6.3.2 Manual runs	75

6.3.1 Using wizards

Wizards provide direct entry 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 the Plus Package is installed, wizards are also available for concentration analysis and customdesigned assays. Settings for a wizard run can be saved in a wizard template.

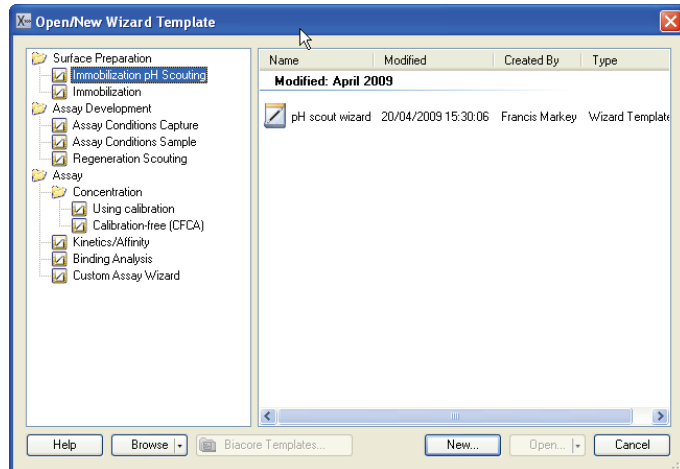
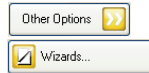
Wizard	Corresponding workflow step
Immobilization pH Scouting	Find immobilization pH
Immobilization	Immobilization
Assay Conditions Capture	Find capture conditions
Assay Conditions Sample	Find sample conditions
Regeneration Scouting	Find regeneration conditions
Concentration Analysis Using calibration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

Step	Action
------	--------

- | | |
|---|---|
| 1 | Click Other Options>> to expand the choices. |
|---|---|

Step	Action
------	--------

- | | |
|---|---|
| 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 wizards 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**. Only templates corresponding to the currently selected wizard type are shown. You may also click **Browse** to open templates stored as files outside the Biacore X100 database. For the Custom Assay Wizard, click **Biacore Templates** to access templates provided with the software installation.

- | | |
|---|--|
| 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.

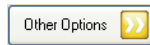
6.3.2 Manual runs

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.

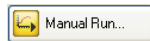
The results from a manual run are saved as a Results item, and can be evaluated in the Evaluation Software. The results cannot however be evaluated with the tools for concentration, kinetics/affinity and binding analysis. It is only possible to view sensorgrams and create plots.

Step	Action
------	--------

- | | |
|---|---|
| 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.

7 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

In this chapter

Section		See page
7.1	Starting the Evaluation Software	77
7.2	Evaluation Software – general features	78
7.3	Presenting data	85
7.4	Workflow for assay evaluation	95

7.1 Starting the Evaluation Software

The Evaluation Software is started automatically when an assay run is completed, or when a saved evaluation item is opened in the Control Software (see [Opening, evaluating or deleting items, on page 63](#)). 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 6.1.1 Logging inlog in to Biacore X100 Control Software, on page 58](#) 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.

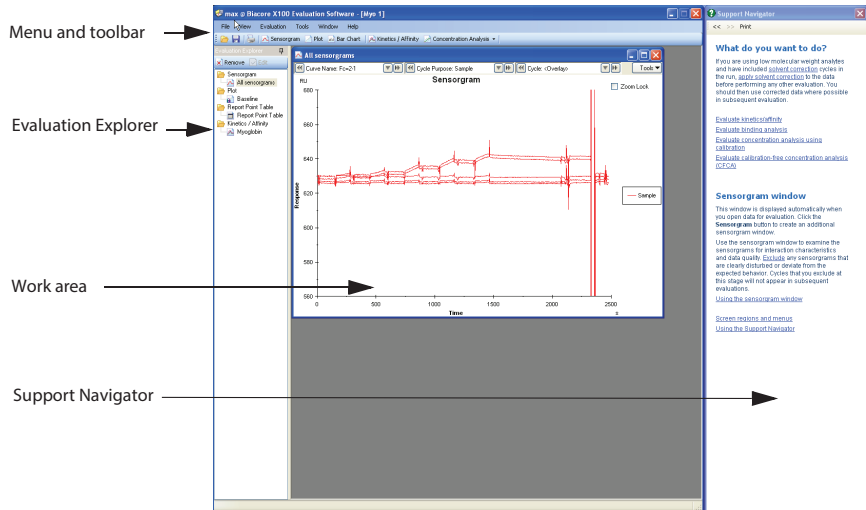
7.2 Evaluation Software – general features

In this section

Section	See page
7.2.1 Start view	79
7.2.2 Opening items	80
7.2.3 Combining results from multiple runs	81
7.2.4 The Evaluation explorer	82
7.2.5 Adding new evaluation items	83
7.2.6 Right-click menus in the work area	84

7.2.1 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 6.1.3 Support Navigator, on page 60](#).

7.2.2 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 6.1.4 Database contents and display, on page 61](#)) to find items.

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 keywords and report points in the results.

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 keywords and report points in a run also define which evaluations that can be performed.

Note: *If you are using solvent correction for small molecule work ([Section 8.5 Solvent correction, on page 104](#)), the predefined plots will by default show uncorrected data. Apply solvent correction before examining the plots, and make sure you examine corrected data where this is relevant.*

7.2.3 Combining results from multiple runs

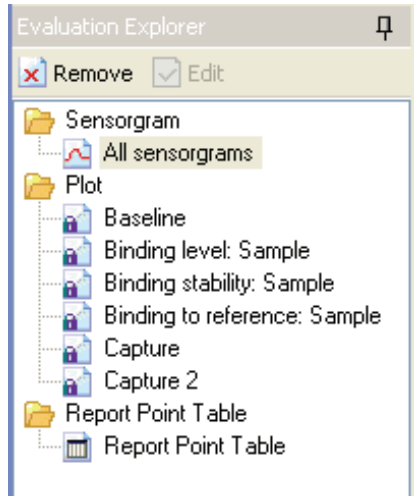
Opening an item with **File** → **Open** will automatically close any current evaluation. To combine results from multiple runs in the same session, use the **File** → **Append Result** option. You can use this feature for example to extend the number of samples that can be analyzed with a single calibration curve for concentration measurement or a single solvent correction curve for small molecule work.



Note: *There are no restrictions in the software for the type of runs that can be combined with **Append Result**. Take care only to combine runs that are performed under equivalent conditions, particularly if you intend to apply calibration or solvent correction data from one run to results from another. Including replicates of the same sample in each run is recommended to provide a control for the consistency of results from different runs.*

Appending results to an evaluation session will delete all the user-defined evaluation items in the session.

7.2.4 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.

7.2.5 Adding new evaluation items

New evaluation items (sensorgrams, plots, bar charts 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.

7.2.6 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.

7.3 Presenting data

All types of results can be displayed in sensorgram and plot windows and as bar charts using the **Bar chart** tool ([Section 7.3.3 Bar charts, on page 88](#)).

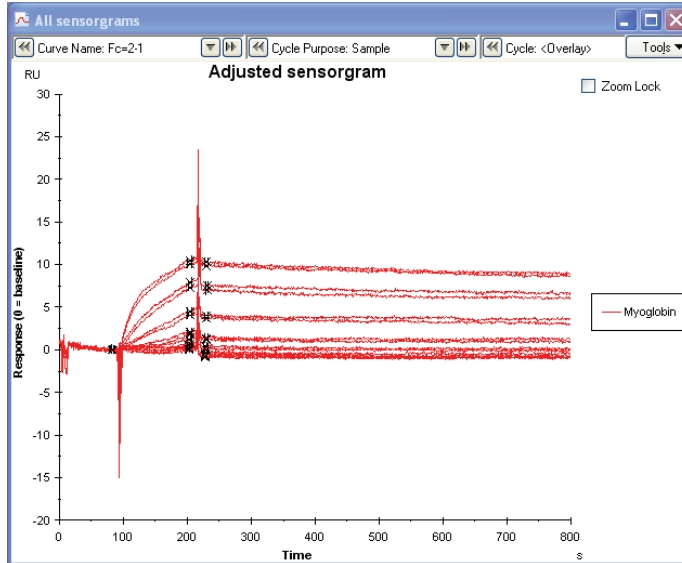
This section gives an introductory overview of the presentation functions. More details may be found in the Support Navigator.

In this section

Section	See page
7.3.1 Sensorgram windows	86
7.3.2 Plot windows	87
7.3.3 Bar charts	88
7.3.4 Kinetics/Affinity analysis	89
7.3.5 Concentration analysis	91
7.3.6 Solvent correction	93
7.3.7 Other evaluation functions	94

7.3.1 Sensorgram windows

Sensorgram windows display the results as sensorgrams.



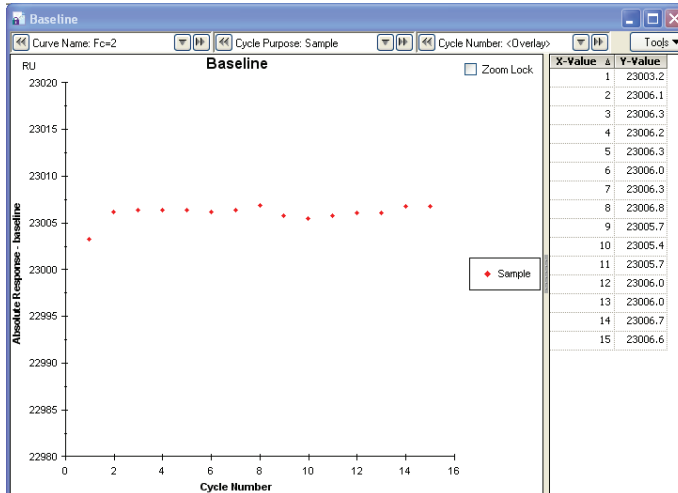
Display functions include

- 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.

7.3.2 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.



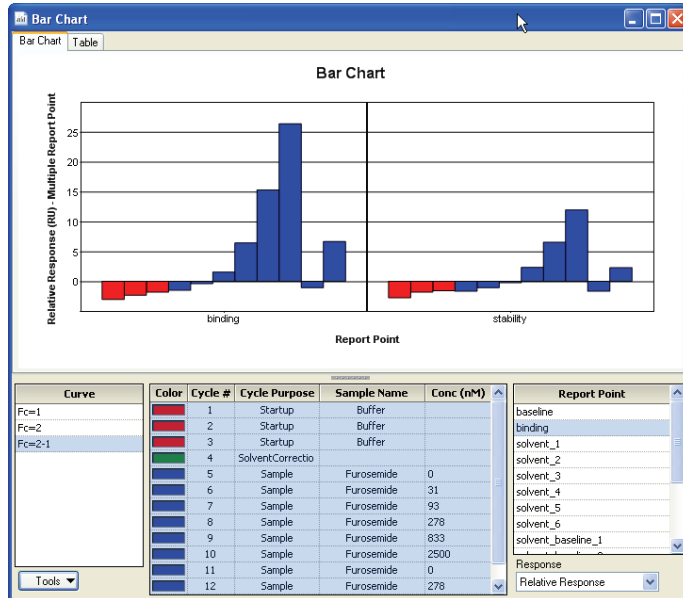
If desired, lines can be fitted to the points in a plot window using either a linear or curved fitting function.

A set of predefined plots is created automatically when results are opened, according to the report points available in the results (see [Section 7.2.2 Opening items, on page 80](#)). These plots are intended in the first place for quality control of the data.

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

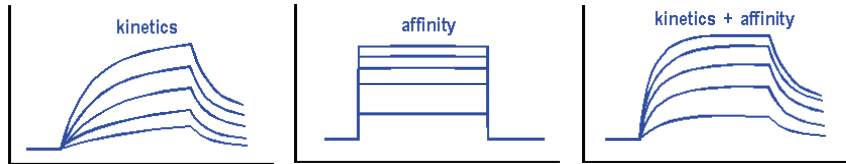
7.3.3 Bar charts

The **Bar Chart** tool creates a bar chart presentation of report point values. Bars can be grouped and colored by various criteria, such as cycles and report points.



7.3.4 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.



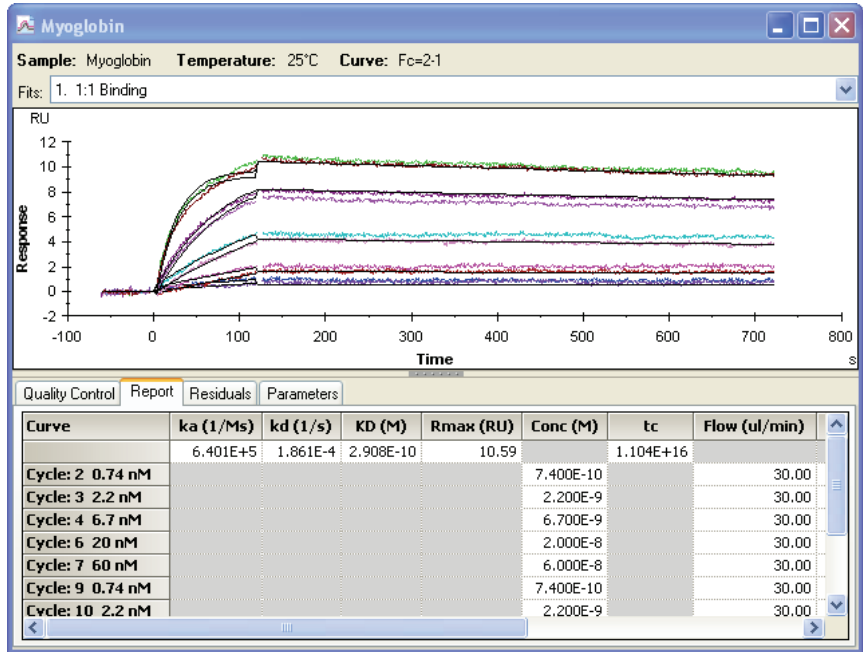
The time required to approach steady state is a function of the dissociation rate constant and the ratio between analyte concentration and affinity constant. The Table below lists the time required to reach 90% of steady state for different values of the rate constant k_d and analyte concentration expressed as a multiple of the affinity constant K_D . From this table it is clear that true steady state affinity determinations are only achievable for dissociation rate constants faster than about $7 \times 10^{-3} \text{ s}^{-1}$. For the majority of interactions affinity constants are best determined from kinetic data.

Table 7.1: Time in seconds taken to reach 90% of steady state for interactions, tabulated as a function of the dissociation rate constant k_d and the analyte concentration expressed as a multiple of the affinity constant K_D . Values in parentheses represent injection times greater than 3 minutes.

k_d (s^{-1})	$C = 0.1K_D$	$C = K_D$	$C = 10K_D$
1×10^{-2}	(209)	115	21
7×10^{-3}	(299)	165	30
1×10^{-3}	(2093)	(1151)	(209)
1×10^{-4}	(20932)	(11512)	(2093)

Kinetics may be evaluated according to a number of different interaction models as described in [Appendix D Fitting equations and models, on page 146](#). Both single- and multi-cycle runs are evaluated with the same tool, using the same fitting models.

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.



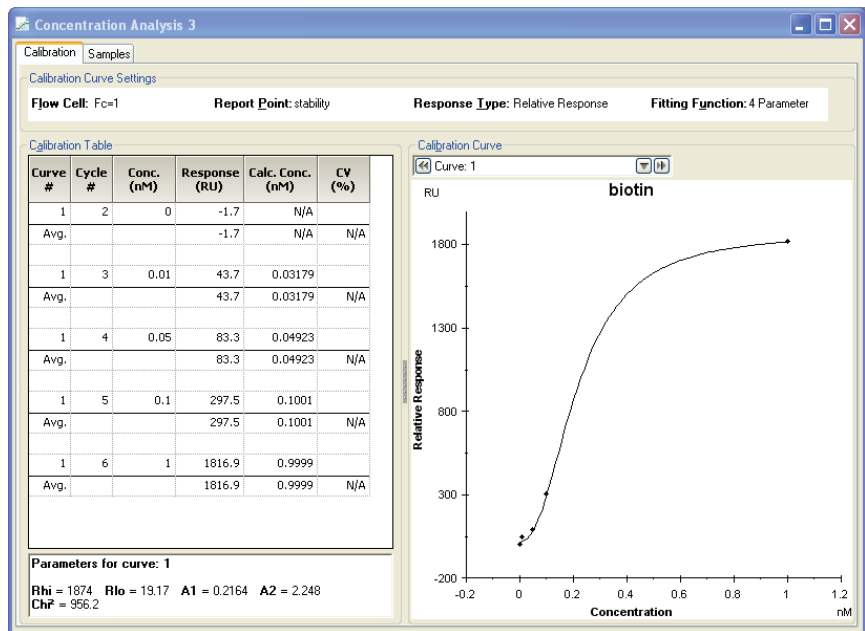
7.3.5 Concentration analysis

To evaluate concentration measurements, click on the **Concentration Analysis** button on the toolbar and choose the type of data to evaluate. You cannot evaluate concentration measurements using calibration with the CFCA evaluation tool or vice versa.

Using calibration

The **Concentration Analysis** → **Using calibration** 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.

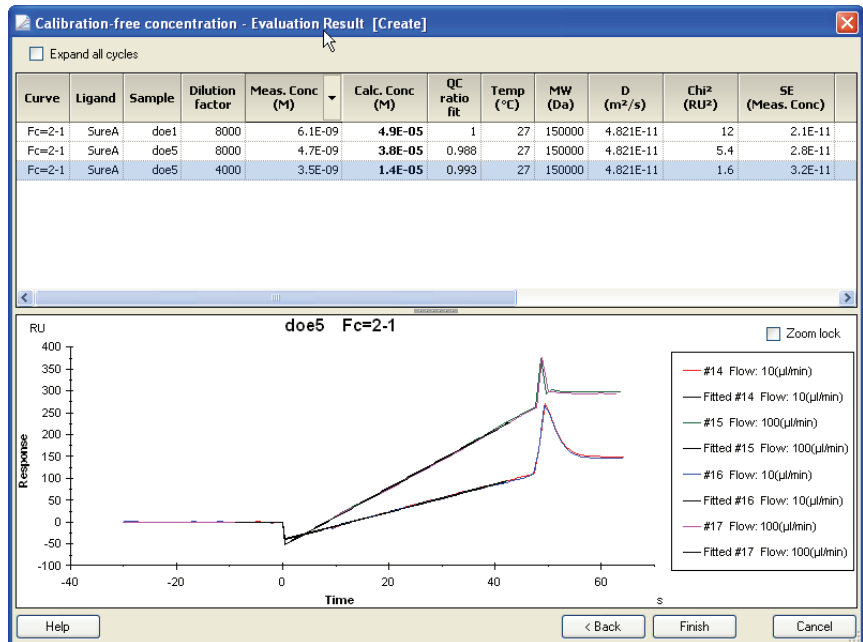


Calibration-free (CFCA)

Calibration-free concentration analysis determines analyte concentration from the initial rate of binding to the surface under diffusion-limited conditions. The evaluation is performed in two steps:

Step Action

- 1 Examine the data and exclude cycles that are not appropriate for reliable concentration determination, on the basis of quality assessment parameters provided in the software (described in detail in the Support Navigator). Exclude unsuitable data.
- 2 Determine the initial binding rate for each sample injection by fitting the data to an interaction model and calculate analyte concentrations using the initial rate and the physicochemical properties (diffusion coefficient and molecular weight) of the analyte.



7.3.6 Solvent correction

Many low molecular weight compounds such as drug candidates are sparingly soluble in water, and organic solvents (commonly DMSO) need to be included in samples to maintain solubility at the required concentrations. These solvents give high bulk responses that can distort the intrinsically low binding responses obtained from low molecular weight analytes. Solvent correction, available in the Custom Assay wizard when the optional Plus Package is installed, corrects for variations in bulk contribution from the solvent and can improve the robustness of the interaction analysis. The principles of solvent correction are described in more detail in [Appendix E Solvent correction, on page 172](#).

To use this function, solvent correction cycles (consisting of a series of injections of different DMSO concentrations) must be included in the run. Biacore templates including solvent correction for various applications are provided with the software as examples.

In the evaluation software, choose **Evaluation** → **Add Solvent Correction** from the main menu to apply solvent correction as the first step before evaluating the results. Corrected data will then be available as an alternative **Curve** selection (e.g. corrected reference subtracted data is listed as **Fc=2-1 corr**), and can be used for evaluation wherever appropriate.

7.3.7 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 thirdparty software if required.

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 evaluation purposes.

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 values for the sample concentration (either in molar units, or in weight units with a value for the molecular weight). Cycles that are to be evaluated as part of the same sample series must have the same sample name.
- 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.*

7.4 Workflow for assay evaluation

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

Step	Action
1	Use Evaluation → Add Solvent Correction to apply solvent correction if you are working with small molecules and have included solvent correction cycles in your assay (see Appendix E Solvent correction, on page 172). In general, use solvent corrected data for subsequent evaluation unless you have a good reason for not doing so.
2	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 7.3.4 Kinetics/Affinity analysis, on page 89), 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.
3	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.
4	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.
5	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.
6	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.
7	Assess your evaluation results in relation to the purpose of the experiment, and repeat or refine the evaluation if required.
8	Save the evaluation session and close the Evaluation Software.

8 Biacore X100 Plus Package

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

- In-line degasser
- Variable analysis temperature control (4–40° C)
- Concentration Analysis wizards for calibrated and calibration-free assays
- Custom Assay Wizard for user-defined assays including support for solvent correction
- Custom immobilization
- User-defined fitting models for kinetics and affinity

In this chapter

Section	See page
8.1 In-line degasser	97
8.2 Variable analysis temperature control	98
8.3 Custom assay wizard	99
8.4 Concentration analysis wizards	101
8.5 Solvent correction	104
8.6 Custom immobilization	105
8.7 User-defined fitting models for kinetics and affinity	106

8.1 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 [Appendix C.3 Pump compartment, on page 145](#)). 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.

8.2 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.

Step	Action
------	--------

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.
---	--

Online - COM1	Current temp: 24.89 °C	Sensor chip: CM5
		Running standby, remaining time: 4.0 days

Online - COM1	Set temperature: 25 °C	Sensor chip: CM5
		Running standby, remaining time: 4.0 days

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

8.3 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.

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 use an existing Custom Assay template

Step	Action
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 Cytiva. 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

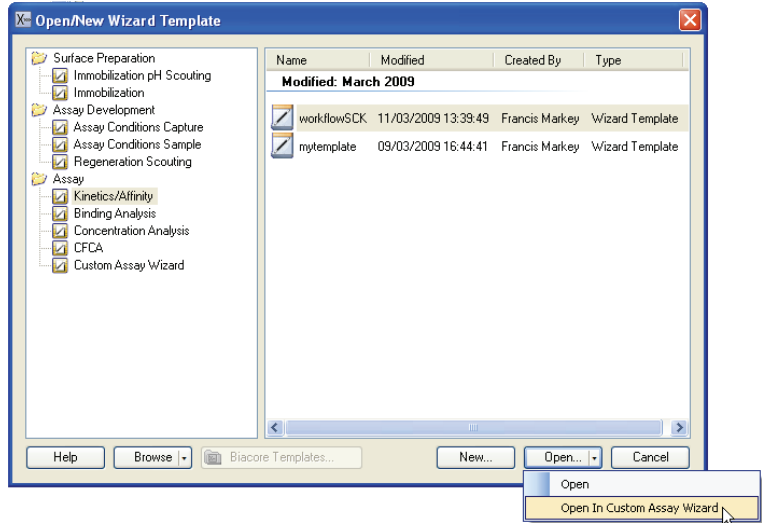
To use an existing template from another wizard

You can open a template from any wizard except Immobilization in the Custom Assay Wizard, allowing customized modifications to wizard-based run settings.

Step	Action
1	Click Other Options → Wizards and select the wizard type for the template you wish to open. Only templates for the chosen wizard type are displayed.
2	Select the template file you wish to open.

Step Action

- 3 Click the arrowhead on the **Open** button and choose **Open In Custom Assay Wizard**.



8.4 Concentration analysis wizards

In this section

Section	See page
8.4.1 Using calibration	102
8.4.2 Calibration-free-concentration analysis (CFCA)	103

8.4.1 Using calibration

Use this wizard to measure analyte concentrations in samples, using known sample to create a calibration curve. Sample concentrations are determined in relation to the concentration in the calibrants: if the calibrant concentration is incorrectly specified, the sample concentrations will be correspondingly incorrect. In this context it is worth remembering that all determinations with Biacore systems provide information on the *fraction of analyte molecules that are capable of binding to the ligand* (the "active concentration"). The concentration of calibrants is usually determined by independent methods, that in many cases measure total concentration regardless of the ligand binding activity. This can introduce systematic errors in the sample concentrations determined in Biacore systems, as illustrated in the Table below. Calibration-free concentration analysis provides an alternative approach that does not rely on correlation with calibrant concentrations.

Table 8.1: Consequences of calibrated "active concentration" measurement with Biacore systems. The example here illustrates the results for samples that are 100% and 50% active respectively, measured against a calibrant that is 80% active. Note that in most real situations the fraction of active material in both calibrants and samples is unknown.

	Calibrant 80% active	Sample 100% active	Sample 50% active
Calibrant concentration	1 nM	–	–
True active concentration	0.8 nM	0.8 nM	0.8 nM
Reported concentration	–	1 nM	1 nM
True total concentration	1 nM	0.8 nM	1.6 nM

To set up a calibrated concentration analysis assay, click **Other Options** → **Wizards** and select **Using calibration** from the **Concentration Analysis** section. Detailed support is found in the Support Navigator.

8.4.2 Calibration-free-concentration analysis (CFCA)

Calibration-free concentration analysis relies on measurement of analyte binding rates under conditions where the rate is limited by the diffusion properties of the analyte molecule, and is independent of the kinetics of interaction with the ligand. Analyte concentrations are calculated from the measured rates and a knowledge of the molecular weight and diffusion coefficient of the analyte (provided as parameters in the assay).

This approach provides absolute values for the active concentration of analyte in the sample, subject to the validity of the physical parameter values and certain assumptions about the dimensions of the flow cell and properties of the sensor surface (these parameters are given internally in the software). The great advantage of CFCA over a calibrated approach is that the results are not dependent on the quality of the calibration standards. The method can also be used for measurement of analytes for which no calibration standards are available.

CFCA can only be used with Sensor Chip CM5 and for analytes with molecular weights of 5000 Da or greater. The dynamic range of the assay is limited to approximately 0.5–50 nM. For unknown samples, measurement of a series of dilutions is recommended to increase the chance of obtaining results within the dynamic range.

To set up a CFCA assay, click **Other Options** → **Wizards** and select **Calibration-free (CFCA)** from the **Concentration Analysis** section. Detailed support is found in the Support Navigator

8.5 Solvent correction

Solvent correction helps to eliminate inaccuracies in response measurements for low molecular weight analytes in the presence of organic solvents (commonly DMSO, often required to maintain solubility for small organic molecules such as drug candidates). Small molecules give intrinsically low response values, which can be masked by the high bulk refractive index from DMSO. Solvent correction factors are determined from special solvent correction cycles supported in the Custom Assay Wizard and are evaluated and applied with functions included in the Evaluation Software when the Biacore X100 Plus Package is installed.

The principles and application of solvent correction are described in detail in [Appendix E Solvent correction, on page 172](#) and in the Support Navigator

8.6 Custom immobilization

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

Step	Action
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.

8.7 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 Fitting equations and models, on page 146](#) in this handbook and the Support Navigator in the software.

9 Maintenance

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

If the instrument has been exposed to biohazards, please contact your local Service representative for further information about decontamination procedures.



NOTICE

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.



NOTICE

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

In this chapter

Section		See page
9.1	User maintenance operations	108
9.2	Standby	111
9.3	Shutdown	112

9.1 User maintenance operations

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.

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

See the table below for a list of maintenance operations. See other sections in this chapter for topics not covered in the table.

Interval	Action	Required material
Daily/after each run	Empty the waste bottle.	-
Weekly	Clean the flow system. ¹ 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 Checking for leaks, on page 109 .	-
Monthly	Clean and disinfect the flow system. Insert a Sensor Chip Maintenance, select Tools → More Tools → Sanitize and follow the instructions on screens.	BIAdesorb solution 1 and 2, disinfectant solution ² , Sensor Chip Maintenance, deionized water (filtered 0.22 µm)
	Perform a System Check and Pump Calibration . Insert a new Sensor Chip CM5, select Tools → More Tools → Pump Calibration and follow the instructions on screens.	Biacore test solution, HBS-EP+ buffer, a new Sensor Chip CM5
	Inspect the needle and needle wash station, look for accumulations of salt or protein.	-

Interval	Action	Required material
	Clean the instrument cover. See <i>Cleaning the instrument cover, on page 110.</i>	Deionized water (filtered 0.22 µm), mild detergent, lint-free cloths

- ¹ 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.
- ² Sodium hypochlorite (0.6% to 1.0% active chlorine) is recommended as disinfectant solution. Instructions for preparing this solution from commercial sodium hypochlorite solutions are given in BIAMaintenance Kit. Do not use hydrogen peroxide-based disinfectants or other products that release gases on use.

Cleaning the needle and wash station



WARNING

The injection needle is sharp. Take care when working in the sample compartment.

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 a hex 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.

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:

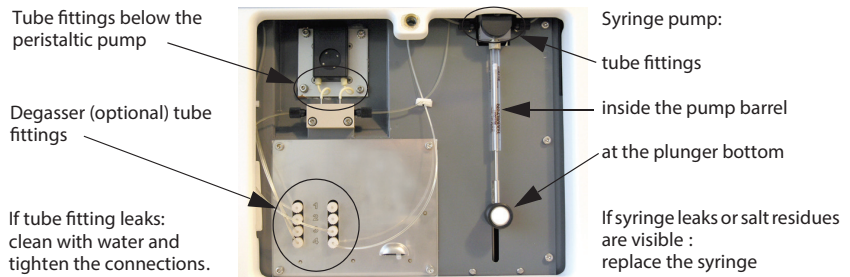


Figure 9.1: Positions to check for leakage

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.

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. Service contracts covering preventive maintenance are available from Cytiva.

9.2 Standby

Biacore X100 is automatically placed in standby mode 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. Standby can run uninterrupted for up to 7 days.

If you intend to leave the instrument for up to 7 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 7 days, after checking the above list.

Note: *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 7 days. However, if you start a new run, change to a buffer fresh for the day.

Starting standby manually

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

9.3 Shutdown

If the instrument is to be left unused for more than 7 days or if the instrument will be moved, perform a shutdown.

It is recommended to perform a **Desorb and sanitize** procedure (see [Section 9.1 User maintenance operations, on page 108](#)) and, if required, also a **Clean needle and needle wash station** procedure (see [Cleaning the needle and wash station, on page 109](#)) 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

Step	Action
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 flow system 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 the figure below.

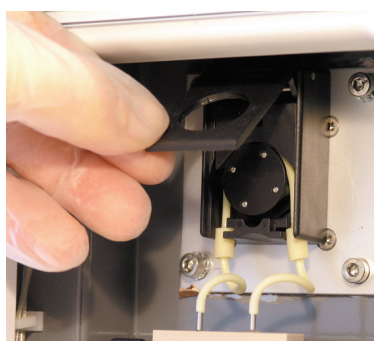


Figure 9.2: Opening the cover of the peristaltic pump to release the clamping of the pump tube. The cover is found behind the lower front door.

Step	Action
3	Exit from Biacore X100 Control Software if it is still running.
4	<p>Remove the bottles, and the buffer bottle cap assembly. Clean the bottle trays if required. Insert the buffer tubes in an empty bottle.</p> <p>The chip is undocked during the Shutdown procedure. An undocked chip is indicated with a flashing sensor chip lamp.</p> <p>You may remove the chip if desired: pull out the chip slide behind the upper front door and lift out the chip.</p>

10 Troubleshooting

This chapter provides information to assist users and service personnel to identify and correct problems that may occur when operating the Biacore X100 instrument.

To avoid problems, follow the instructions and recommendations given in this Handbook, and clean and maintain the instrument regularly as described in [Chapter 9 Maintenance, on page 107](#). If the suggested actions in this guide do not solve the problem, or if the problem is not covered by this guide, contact your Cytiva representative for advice.

In this chapter

Section		See page
10.1	Instrument related troubleshooting	115
10.2	Assay related troubleshooting	119

10.1 Instrument related troubleshooting

See table below for a list of problems. If you do not find your problem search continue searching in this section or contact you local Cytiva representative.

Error description	Corrective action
Proteins and other substances adsorb to the walls of the flow system, giving carry-over problems between assays.	Clean the flow system with Desorb more often than once a week. For more information see Section 9.1 User maintenance operations, on page 108 .
Carry-over problems persist after Desorb and Sanitize.	Run Tools → More Tools → Super-clean. Superclean, on page 118 .
Salt residues or other deposits have accumulated on the needle or needle wash station.	Clean the needle and wash station. For more information see Section 9.1 User maintenance operations, on page 108 .
The needle is obstructed or damaged.	Replace the needle (Tools → More Tools → Replace Needle). For more information see Damaged needle, on page 115 .
Sample consumption is unexpectedly low, sensorgrams are irregular.	Run Tools → More Tools → Flow system wash .
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).

Damaged needle

Cause	Action
Wrong caps have been used on the vials.	Ensure that only caps supplied by Cytiva are used to cap the 1.5 ml sample and reagent vials. Never cap the water vial. Change needle as described below.
Wrong vials have been used.	Ensure that only recommended vials are used, see Appendix B.4 Sample rack, on page 133 . Change needle as described below.

Cause	Action
Rack movement was obstructed during a run.	Change needle as described below.
The rack mechanism does not function properly.	Contact your local Service representative.

Step	Action
1	To replace the needle, select Tools → More Tools → Replace Needle and follow the instructions on the screen.
2	When the needle replacement and calibration is finished, start the standby by clicking Tools → Standby .

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:

Step	Action
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.

The table below shows troubleshooting options if System Check and Pump Calibration fails.

Failed test	Actions
A: Peristaltic pump calibration	<p>Check that the pump cover is properly closed.</p> <p>Check that the buffer tubes reach the buffer liquid and that they are not squeezed.</p> <p>Check that the pump tube does not leak nor has been punctuated by mistake.</p> <p>If a new Peristaltic pump calibration still fails, change the pump tube.</p>

Failed test	Actions
B: Response	<p>If the Fc-values in the results are too low, check that you used a non-immobilized Sensor Chip CM5.</p> <p>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 (Biacore test solution).</p>
C: Injections	<p>Check that the tubes do not leak.</p> <p>Contact your local Service representative.</p>
D: Noise	<p>Check that you used a non-immobilized Sensor Chip CM5.</p> <p>Check that the temperature stability is not affected by inadequate ventilation, sun exposure or similar.</p>

Troubleshooting for Liquid flow problems

	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 Checking for leaks, on page 109 .
Air is injected during sample or reagent injections.	Insufficient volumes of sample and/or reagents.	<p>Ensure that you use the volumes specified in the software for the run. Larger volumes may be needed if the problem persists.</p> <p>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.</p> <p>Perform a System Check and Pump Calibration to calibrate the peristaltic pump.</p>
Samples become crosscontaminated.	Not enough water to clean the injection needle.	Always fill the water vial in the rack before starting the run.

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 instead of guanidine-HCl and HCl respectively.

Troubleshooting for 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.

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.

Troubleshooting for software issues

In the case of software malfunction or other software issues, 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.

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 [System check and pump calibration, on page 116](#)), then contact your local Service representative.

10.2 Assay related troubleshooting

The Support Navigator in the software provides help on assay related problems. If you cannot solve your problems with the information provided, contact your local Application Support specialist.

Appendix A

Installation requirements

In this chapter

Section		See page
A.1	General guidelines	121
A.2	Space requirements	122
A.3	Mains power supply	123
A.4	Heating and ventilation	124
A.5	Moving the instrument within the lab	125

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 [Appendix A.2 Space requirements, on page 122](#) to [Appendix A.4 Heating and ventilation, on page 124](#). If you need to move the instrument after installation, follow the instructions in [Appendix A.5 Moving the instrument moving the instrument within the lab, on page 125](#).

A.2 Space requirements

The size of the instrument is indicated in the Figure below. 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.

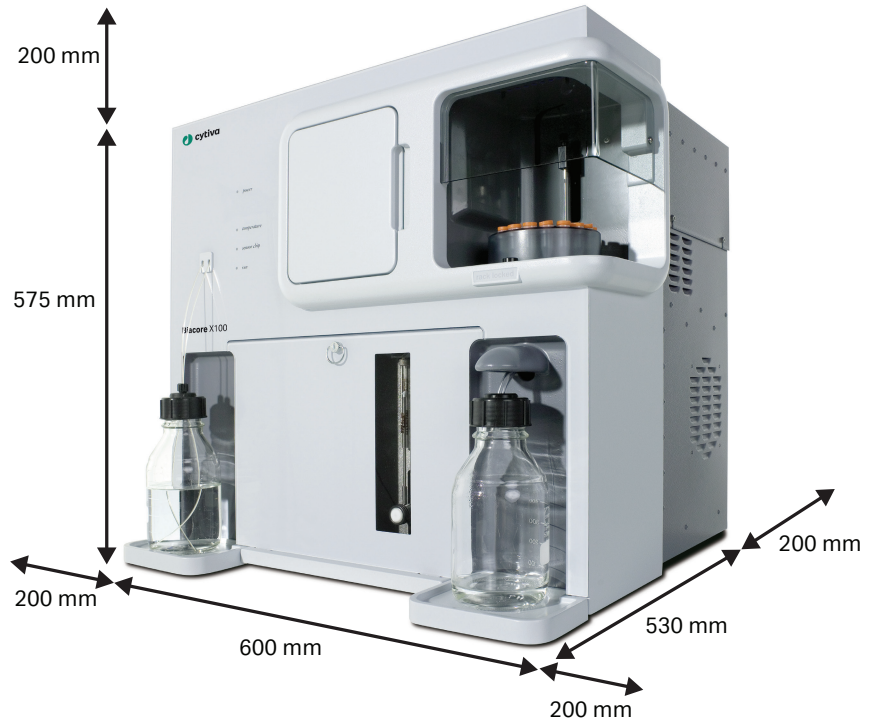


Figure 11.1: Space requirements for Biacore X100 instrument.

A.3 Mains power supply

The instrument and the PC require mains power outlets with protective earth as specified in the table below.

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

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



NOTICE

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

In this section

Section	See page
A.5.1 Shutting down the system	126
A.5.2 Connecting to mains power	127
A.5.3 Connecting the instrument to the PC	128

A.5.1 Shutting down the system

Before moving the system within the lab:

Step	Action
1	Shut down the instrument completely (See Section 9.3 Shutdown, on page 112).
2	Shut down the computer.
3	Disconnect the serial communication cable.
4	Carefully lift the instrument by 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 Appendix A.2 Space requirements, on page 122 to Appendix A.4 Heating and ventilation, on page 124 .

A.5.2 Connecting to mains power



WARNING

Do not turn on the mains power switches before all connections are made.

Step	Action
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.

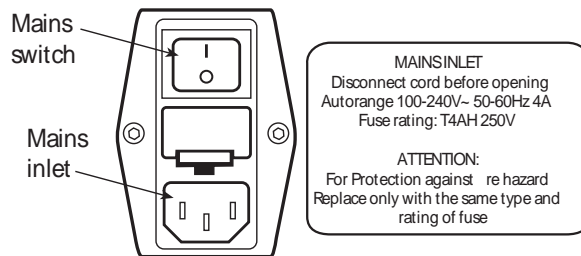


Figure 11.2: Mains panel at the rear of the instrument.

A.5.3 Connecting the instrument to the PC

Connect a serial communication cable between the **COM1** (or **IO1O1A**) 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.

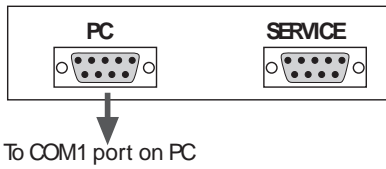


Figure 11.3: Computer ports on the rear of the instrument.

Appendix B

Specifications

In this chapter

Section	See page
B.1 General	130
B.2 Instrument	131
B.3 System controller and software	132
B.4 Sample rack	133
B.5 Liquid containers provided	134
B.6 Chemical resistance	135

B.1 General

Automation	Maximum 15 samples, 24h unattended operation
Molecular weight detection	Down to 100 Da in various sample environments
Required sample volume	Injection volume + 20-30 μ l (application dependent)
Sample/reagent capacity	15 sample/reagent vials, volume 1.5 ml 1 water vial, volume 4 ml
Analysis time per sample	Typically 5-15 min
Detection limit	Typically 0.1 nM for >10 000 Da analytes. Typically 1 nM for <10 000 Da analytes.
Typical range for kinetics:	
Association rate constant (k_a):	10^3 – 10^7 $M^{-1}s^{-1}$ (for typical protein-protein interactions)
Dissociation rate constant (k_d):	10^{-5} – $0.1 s^{-1}$
Number of flow cells	2
Flow cell height	Approximately 0.05 mm
Flow cell volume	Approximately 0.06 μ l

B.2 Instrument

Dimensions (see also Fig. 11.1, on page 122)	
W×D×H	600 × 530 × 575 mm
Net weight	Total: 47 kg
Mains requirements	Processing Unit and System Controller: Autorange 100–240 V ac, frequency 50/60 Hz, protective earthing
Fuses	2 × T4.0AH 250V
Power consumption	Processing Unit: max. 400 VA
Ambient temperature range	18–33°C
Ambient humidity	≤80% RH
Analysis temperature range	Without Biacore X100 Plus Package: 25°C. With Biacore X100 Plus Package: 4–40°C (maximum 10 degrees below ambient temperature).
Safety standard, EMC	See the <i>Biacore X100 Operating Instructions</i> .

B.3 System controller and software

PC operating system	Windows 10 or 11, Professional or Enterprise, 64-bit, English versions
Database (included)	SQL Server 2022 Express
Power consumption	See manufacturer's manual

B.4 Sample rack

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

¹ Use only vials and caps from Cytiva.

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. The Table 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

Table 11.1: Chemical resistance.

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

Appendix C

Technical description

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

In this chapter

Section		See page
C.1	Detection principle	137
C.2	Detection system	141
C.3	Pump compartment	145

C.1 Detection principle

In this section

Section		See page
C.1.1	Surface plasmon resonance	138
C.1.2	What SPR measures	140

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.

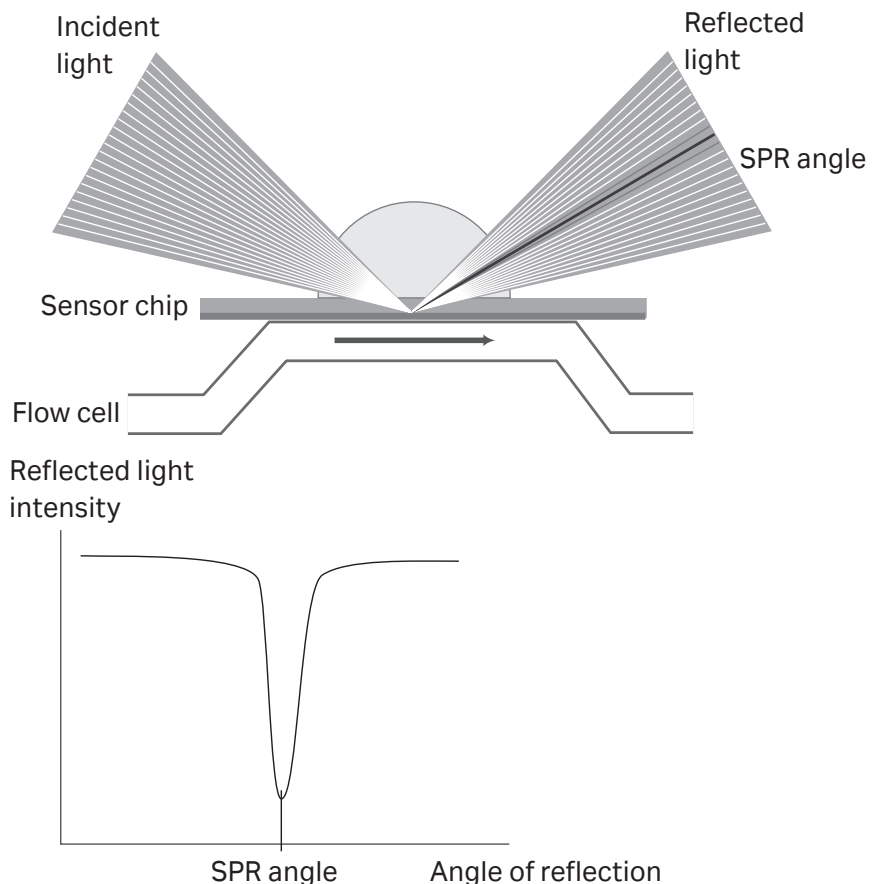


Figure 11.1: The SPR principle.

At a certain combination of angle of incidence and energy (wavelength), the incident light excites *plasmons* (electron charge density waves) in the gold film.

C. Technical description

C.1 Detection principle

C.1.1 Surface plasmon resonance

As a result, a characteristic absorption of energy via the evanescent wave field occurs and results in a drop in the intensity of the reflected light (see the Figure above).

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 for biological molecules 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.

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.

In this section

Section	See page
C.2.1 Sensor chip	142
C.2.2 Integrated μ -Fluidic Cartridge (IFC)	143
C.2.3 Flow cells	144

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, the Figure below shows the sensor chip removed from the cassette.

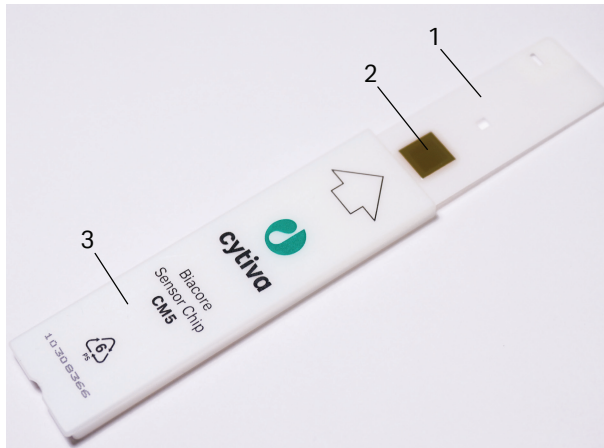


Figure 11.2: The sensor chip and the protective cassette.

Part	Description
1	Frame
2	Sensor surface
3	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 [Appendix C.2.2 Integrated \$\mu\$ -Fluidic Cartridge \(IFC\)](#), on page 143).

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.

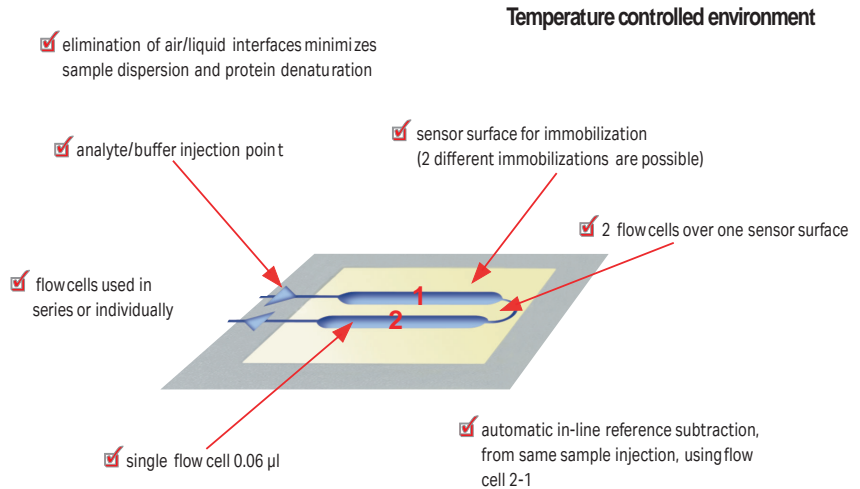


Figure 11.3: Flow cells on the sensor chip.

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.



NOTICE

Do not place anything on the lower front door when it is open.

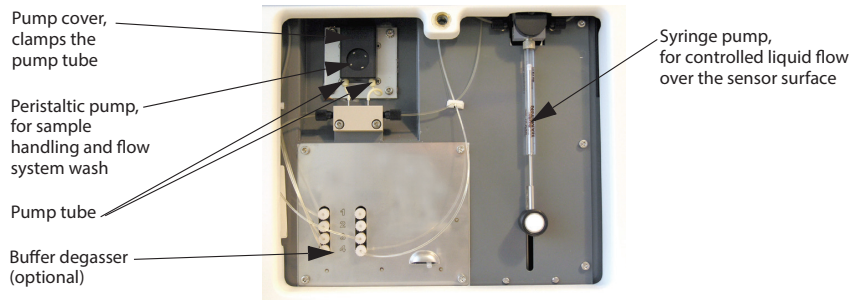


Figure 11.4: The pump compartment behind the pump compartment door.

Appendix D

Fitting equations and models

In this chapter

Section		See page
D.1	Curve fitting principles	147
D.2	Statistical parameters	150
D.3	Predefined models - kinetics and affinity	153
D.4	Creating and editing models	161
D.5	Predefined models - concentration analysis	168

D.1 Curve fitting principles

All kinetic and affinity analysis calculates results by fitting the experimental data to a mathematical model. It is important to realize 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.

Single- and multi-cycle kinetic analyses are evaluated with the same models.

In this section

Section	See page
D.1.1 Fitting procedure	148
D.1.2 Local and global parameters	149

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 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.2 Statistical parameters

In this section

Section		See page
D.2.1	Report points	151
D.2.2	Significance of fitted parameters	152

D.2.1 Report points

Response values in the report point table are calculated as average values over a specified window (usually 5 seconds).

The report point table includes the following statistical functions:

SD	<p>Standard deviation of data points in the time window, calculated as</p> $SD = \sqrt{\frac{1}{(n-1)} \sum (y - \bar{y})^2}$ <p>where n = number of points and y = response in RU</p>
Slope	<p>Slope during time window in RU s⁻¹, calculated as</p> $\text{slope} = \frac{\sum (y - \bar{y})(x - \bar{x})}{\sum (x - \bar{x})^2}$
LRSD	<p>Alignment of the slope to a straight line (regression coefficient), calculated as</p> $LRSD = \sqrt{\frac{Q_0}{(n-2)}}$ <p>where</p> $Q_0 = \sum (y - \bar{y})^2 - \frac{(\sum (y - \bar{y})(x - \bar{x}))^2}{\sum (x - \bar{x})^2}$

D.2.2 Significance of fitted parameters

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 Tvalue 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 $10^8 \text{ RU}\cdot\text{M}^{-1}\text{s}^{-1}$, 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 X100.

D.3 Predefined models - kinetics and affinity

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

In this section

Section	See page
D.3.1 Mass transfer parameters	154
D.3.2 Kinetics – 1:1 binding	155
D.3.3 Kinetics – Bivalent Analyte	156
D.3.4 Kinetics – Heterogeneous Analyte	157
D.3.5 Kinetics – Heterogeneous Ligand	158
D.3.6 Kinetics – Two State Reaction	159
D.3.7 Affinity – Steady State 1:1	160

D.3.1 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 nonturbulent laminar flow that prevail in the Biacore flow cell is characterized by the *mass transfer coefficient* k_m (units $\text{m}\cdot\text{s}^{-1}$):

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

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 $\text{RU}\cdot\text{M}^{-1}\text{s}^{-1}$), obtained by adjusting the mass transfer coefficient approximately for the molecular weight of the analyte and for the conversion of surface concentration to RU:

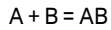
$$k_t = k_m \times \text{MW} \times 10^9$$

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

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

D.3.2 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:

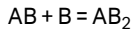
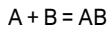


The following parameters are fitted by the model:

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.3.3 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.



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.*

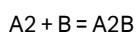
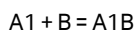
The following parameters are fitted by the model:

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.3.4 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.



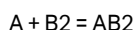
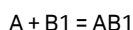
The following parameters are fitted by the model:

ka1	Association rate constant for the first and second analytes
ka2	($M^{-1}s^{-1}$)
kd1	Dissociation rate constant for the first and second analytes (s^{-1})
kd2	
tc1	Flow rate-independent component of the mass transfer constant
tc2	for the first and second analytes
Rmax1	Analyte binding capacity of the surface for the first and second
Rmax2	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.3.5 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.



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

D.3.6 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:



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.3.7 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.4 Creating and editing models

You can create and edit your own models for kinetics of affinity evaluation if you have the Biacore X100 Plus Package.

To create models, 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 [Appendix D.4.1 Interaction models](#) *interaction models for kinetics, on page 162* and equation models in [Appendix D.4.2 Equation models for kinetics and affinity, on page 166](#).

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.

In this section

Section	See page
D.4.1 Interaction models for kinetics	162
D.4.2 Equation models for kinetics and affinity	166

D.4.1 Interaction models for kinetics

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

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.
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 parameter is only used in heterogeneous analyte models. Check the box and enter the molecular weight parameter for the analyte to which the binding capacity parameter refers. Binding capacity for the other analyte will be calculated using the molecular weight values.

Complex

The complex is formed on the surface and generates response and has the properties listed below.

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

Step	Action
------	--------

- | | |
|---|--|
| 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: |
|---|--|

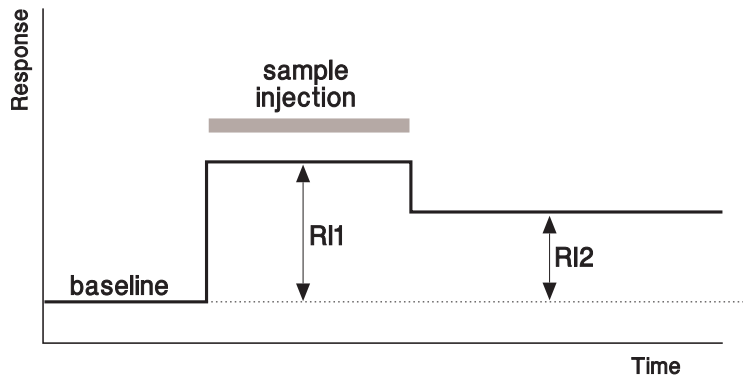
Bulk And Drift

Response (RU)

Refractive index (1): (association)

Refractive index (2): (dissociation)

Drift (in RU):

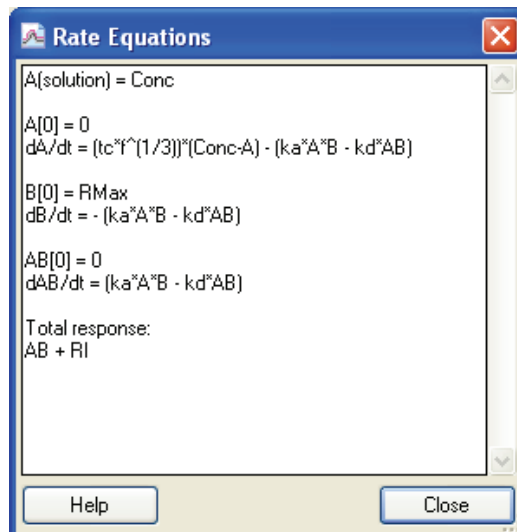


Check the **Drift** box and enter an expression describing the drift (most commonly a linear function of time) to account for baseline drift.

- | | |
|---|---|
| 2 | Enter the reaction scheme in the Reaction panel using the pull-down list for each reactant. Enter parameter names for the forward and backward rate constants for each line in the reaction scheme. (The terms k-forward and kbackward 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. |
|---|---|

Step	Action
------	--------

- | | |
|---|--|
| 3 | <p>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.</p> <p>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.</p> <p>Check Allow negative value if the parameter can be below zero. Enter a description of the parameter for ease of identification.</p> <p>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.</p> <p>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.</p> |
| 4 | Click Rate Equations to display the equations generated by the software. |



D.4.2 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 Req as a function of concentration Conc.

Edit Model

Model Name: Two site affinity

Description: Steady state affinity with two ligand sites

Formula

$$\text{Conc} \cdot \text{Rmax1} / (\text{Conc} + \text{KD1}) + \text{Conc} \cdot \text{Rmax2} / (\text{Conc} + \text{KD2})$$

Independent variable: Conc

Parameters

Name	Type	Initial value	Allow negative value	Description
KD1	Fit global	XMax/10	<input type="checkbox"/>	Affinity for site 1
Rmax1	Fit local	YMax/2	<input checked="" type="checkbox"/>	Rmax for site 1
KD2	Fit global	XMax/10	<input type="checkbox"/>	Affinity for site 2
Rmax2	Fit global	YMax/2	<input checked="" type="checkbox"/>	Rmax for site 2

Add Edit Delete

Report

Name	Value
KD1 (M)	KD1
Rmax1 (RU)	Rmax1
KD2 (M)	KD2
Rmax2 (RU)	Rmax2

Help OK Cancel

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

D. Fitting equations and models

D.4 Creating and editing models

D.4.2 Equation models for kinetics and affinity

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 behavior.*

D.5 Predefined models - concentration analysis

In this section

Section		See page
D.5.1	Using calibration	169
D.5.2	Calibration-free concentration analysis (CFCA)	170

D.5.1 Using calibration

Calibration curves for concentration analysis can be evaluated using linear or 4-parameter equations.

Linear fit

This option fits the curve to a straight line defined by

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

Closeness of fit is reported as the correlation coefficient R2.

4-parameter equation

The 4-parameter equation is a general curve-fitting equation that meets the requirements for most non-linear calibration curves.

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

where y and x are the plot coordinates

R_{hi} and R_{lo} are fitting parameters that correspond to the maximum and minimum response levels respectively

A_1 and A_2 are additional fitting parameters

Closeness of fit for the 4-parameter equation is reported as chi-squared ([Appendix D.1.1 Fitting procedure, on page 148](#)).

D.5.2 Calibration-free concentration analysis (CFCA)

Sensorgrams for CFCA are fitted to a 1:1 interaction model, and the concentration is calculated from the mass transport coefficient obtained from the fitting. Since measurements are made under conditions of mass transport limited binding, the interaction kinetics are irrelevant and the 1:1 model can be used even for interactions that are known to be more complex.

Mass transfer coefficient

The observed binding rate under conditions of limiting mass transfer of analyte to the surface is characterized by the **mass transfer coefficient** k_m (units $\text{m}\cdot\text{s}^{-1}$). This parameter is related to the diffusion coefficient of the analyte and the dimensions of the flow cell by the semi-empirical equation:

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

where D is the diffusion coefficient of the analyte ($\text{m}^2\cdot\text{s}^{-1}$)

f is the flow rate of solution through the flow cell ($\text{m}^3\cdot\text{s}^{-1}$)

h, w, l are the flow cell dimensions (height, width, length in m)

The flow cell dimensions are provided in the evaluation algorithms in the software. The diffusion coefficient is supplied by the user as a parameter for the analysis. Diffusion coefficients may be found in the literature or estimated from the physical properties of the molecule, using the interactive diffusion coefficient calculator tool on cytiva.com/biacore.

Quality control

It is important that the sensorgrams at the lowest and highest flow rates (recommended 10 and 100 $\mu\text{l}/\text{min}$ respectively) are sufficiently separated. If the curves are close together or coincide, this indicates that there is not sufficient mass transport limitation in the binding for reliable concentration measurement. The table column **QC ratio** in the evaluation results provides a guidance value for this assessment: sufficient mass transport limitation is indicated by a value for the QC ratio of about 0.2 or higher. Exclude samples where the ratio is less than this value.

Note: *The QC ratio in the first evaluation dialog is based on the initial rate determined from the sensorgrams in the interval 12–25 seconds after the injection start. If data is removed from this region, the initial rate in the first dialog will be reported as nd (not determined). In the final results, the QC ratio is based on the initial rate determined from the fitted curves, and can thus be reported even if sensorgram data is deleted from the region used for initial rate calculation.*

The QC ratio is calculated as follows:

$$\text{quotient } Q = \frac{\text{initial rate at high flow rate}}{\text{initial rate at low flow rate}} \times \sqrt[3]{\frac{\text{low flow rate}}{\text{high flow rate}}}$$

Under conditions of complete mass transport limitation, the binding rate is proportional to the cube root of the flow rate, so the quotient Q has a value of 1. When there is no mass transport limitation, the binding rate is independent of the flow rate so Q has a value equal to the cube root of the flow rate ratio. The range of possible theoretical values for Q will thus depend on the flow rates used (for flow rates of 10 and 100 $\mu\text{l}/\text{min}$, the minimum value is 0.46). The QC ratio is calculated from the measured value for Q normalized to a scale of 0–1:

$$Q_{\max} = 1$$

$$Q_{\min} = \sqrt[3]{\frac{\text{low flow rate}}{\text{high flow rate}}}$$

$$\text{QC ratio} = \frac{Q_{\text{measured}} - Q_{\min}}{Q_{\max} - Q_{\min}}$$

The recommended acceptance value of 0.2 or higher for the QC ratio is empirically determined.

Occasionally, the measured binding rate may be lower at the higher flow rate, leading to a negative value for the QC ratio. This occurs as a result of experimental variation when the binding rate is not affected by flow rate (so the binding rates should in theory be equal and the QC ratio should be 0). Samples where the QC ratio is negative do not show sufficient mass transport limitation for calibration-free concentration measurements.

Appendix E

Solvent correction

Solvent correction functions are available if the Biacore X100 Plus Package is installed.

In this chapter

Section	See page
E.1 Background	173
E.2 How solvent correction works	174
E.3 When should solvent correction be used?	175
E.4 Applying solvent correction	176

E.1 Background

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

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

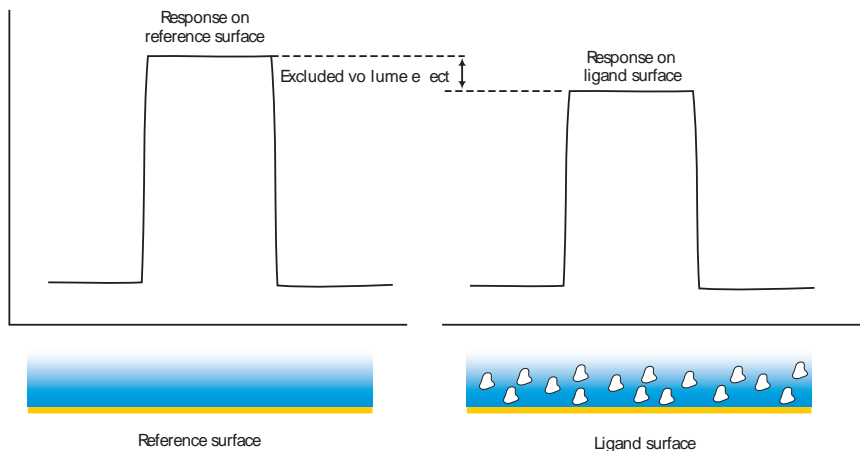


Figure 11.1: Bulk solution is excluded from the volume occupied by ligand molecules on the ligand surface, so the bulk contribution to the relative response is smaller than on the reference surface.

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

Organic solvents like DMSO often give a high bulk response (addition of 1% DMSO gives a bulk response of about 1200 RU), so that small variations in the DMSO content lead to significant variations in the bulk response between samples. Such variations are common in the preparation of diverse samples such as drug candidates for screening applications, and can arise from small volume errors even in careful preparation of dilution series for kinetics and affinity measurement.

E.2 How solvent correction works

Solvent correction factors are determined by injecting a series of blank samples containing a range of solvent concentrations over the active and reference surfaces, and plotting the reference-subtracted response against the relative response on the reference surface. This plot shows the size of the reference subtraction error as a function of the reference response. Each sample measurement is then corrected by a factor obtained by measuring the relative response on the reference surface and reading the corresponding value for the reference-subtraction error from the correction curve (see the Figure below).

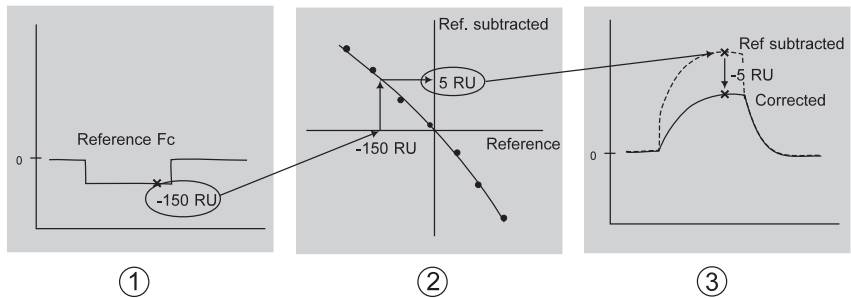


Figure 11.2: The principle of solvent correction. 1. The sensorgram from the reference flow cell shows a bulk displacement (-150 RU in the illustration) during sample injection because the sample and running buffer are not exactly matched. 2. From the solvent correction curve, a displacement of -150 RU in the reference sensorgram corresponds to a solvent error of +5 RU in the reference-subtracted sensorgram. 3. The reference-subtracted sensorgram is corrected by subtracting the solvent error. This procedure is applied to every point during sample injection.

Solvent correction is applied only to response levels during sample injection, since the correction adjusts for differences in the bulk refractive index of the samples. Before and after sample injection, when buffer is flowing over the surface, the bulk response is constant and correction is meaningless.

E.3 When should solvent correction be used?

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

- the expected analyte responses are low (of the order of 50 RU or less),
- the ligand is a macromolecule immobilized at a high density (typically 5,000 RU or more – lower ligand densities lead to excluded volume effects that are too small to merit correction),
- the bulk response on the reference surface is high (of the order of 200 RU or more) and is subject to variation between samples of at least the same order of magnitude as the measured binding response.

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

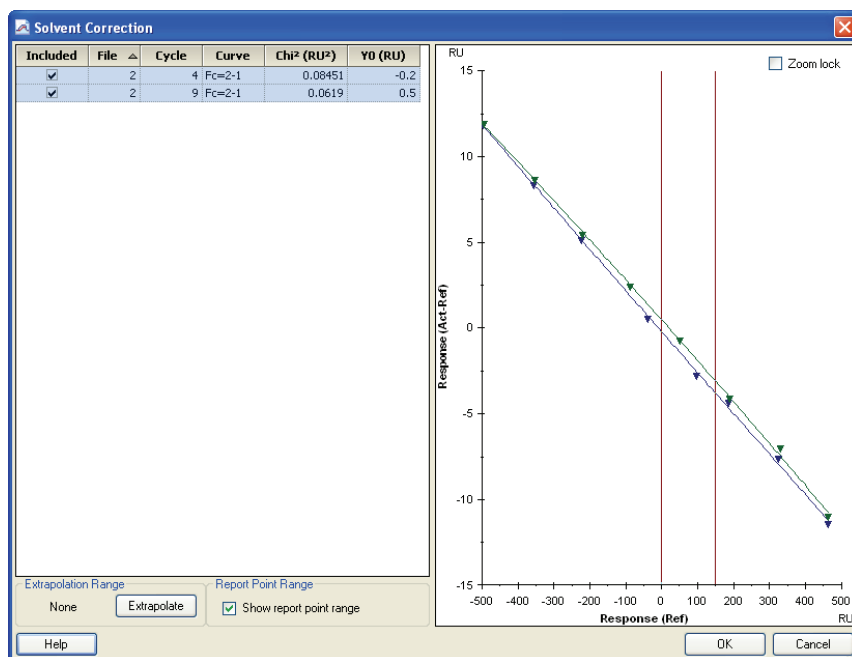
For kinetic assays, solvent correction will in most cases not make any significant difference to the reported kinetic constants, since the reference subtraction error appears as a bulk response which is fitted locally to the **RI** parameter ([Appendix D.3 Predefined models predefined models - kinetics and affinity, on page 153](#)). However, applying solvent correction eliminates this bulk response so that the results may be easier to assess.

For affinity measurements, evaluation is based on the steady state response during sample injection, so that the effect of solvent correction may be significant. This is particularly true for interactions with rapid on and off rates, where the binding kinetics cannot be distinguished from bulk response shifts. In addition, preparation of samples as a dilution series can lead to correlation between solvent mismatch and analyte concentration, adding to the difficulty of distinguishing between bulk response and analyte binding.

As a general recommendation, include solvent correction cycles in the analysis when you are working with small molecules in the presence of DMSO. The decision as to whether it is appropriate to apply solvent correction is made in the evaluation software.

E.4 Applying solvent correction

To apply solvent correction, choose **Add solvent correction** from the **Evaluation** menu. In order to apply solvent correction, the run must include solvent correction cycles (see [Section 8.5 Solvent correction, on page 104](#)).



The left-hand panel of this dialog lists the solvent correction curves in the run, and the corresponding curves are shown in the right-hand panel. The solvent correction curve is fitted to the experimental points using a second-degree equation. Beware of applying solvent correction if the correction curve does not fit the experimental points closely. Scatter in the correction points indicates that the measurements are not reliable, and applying correction derived from such curves can distort the measured responses unnecessarily. For reliable solvent correction, the chi-squared value should be less than 2 RU.

Note: *In judging the quality of the solvent correction data, take note of the y-axis scale in the display. The curves are automatically scaled to fit the window. If the range of solvent correction is small (as in the illustration above), points may appear to scatter widely from the fitted curve without necessarily indicating poor quality.*

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

Click **OK** to apply the solvent correction. Correction will be applied to the sample injection phase(s) of all sensorgrams. Any data points that lie outside the correction range will be discarded and the corresponding sensorgram will contain gaps corresponding to the invalid data.

Appendix F

System administration

In this chapter

Section		See page
F.1	Database overview	179
F.2	Biacore X100 user types	180
F.3	User administration	181
F.4	Database backup	182
F.5	Restoring a database	183
F.6	Database connections	184
F.7	Re-installing the Biacore X100 software	188

F.1 Database overview

Biacore X100 saves all data in an SQL Server 2022 Express database. The database is installed on the system controller during the software installation. This local database can also be accessed by other computers via a network.

F.2 Biacore X100 user types

There are three types of users in Biacore X100:


- **BiacoreX100Users** can create runs, methods and evaluations. They can also create new sub-folders in their own user folder and view all other users' data, but only delete their own data.
- **BiacoreX100Administrators** can delete users and other users' data, as well as everything that **BiacoreX100Users** can do.
- **SQLServerX100DatabaseAdministrators** can do the same as **BiacoreX100Administrators** as well as restore databases through Biacore X100 Backup and Restore.

When installing a local Biacore X100 database for the first time, all authenticated Windows users becomes **BiacoreX100Administrators** and **SQLServerX100DatabaseAdministrators**. It is recommended to change these settings, refer to the *Biacore X100 v2.1 Installation Instruction 29288193* for details.

F.3 User administration

In Biacore X100 software v2.1 and later, users are logged in with their Windows or Active Directory user credentials. Users are added to the Biacore X100 database upon their first login. Data produced in previous software versions are still available in the database, but are associated with old user accounts. This means that only **BiacoreX100Administrators** can delete such data.

Note: *To see data generated by user accounts from Biacore X100 software v2.0.3 or earlier, the old database must first be migrated to the v2.1 database.*

BiacoreX100Administrators can delete users that do not have any associated data stored in the database, such as runs or evaluations. To delete a user as an administrator, select the user and click **Delete selected folder or user** () in the **Quick Filter** tab.

Changing the user type of Biacore X100 users can be done from the Computer Management application in Windows operating system by adding or removing user accounts to the different groups in **Local Users and Groups/Groups** (requires Windows administrator rights).

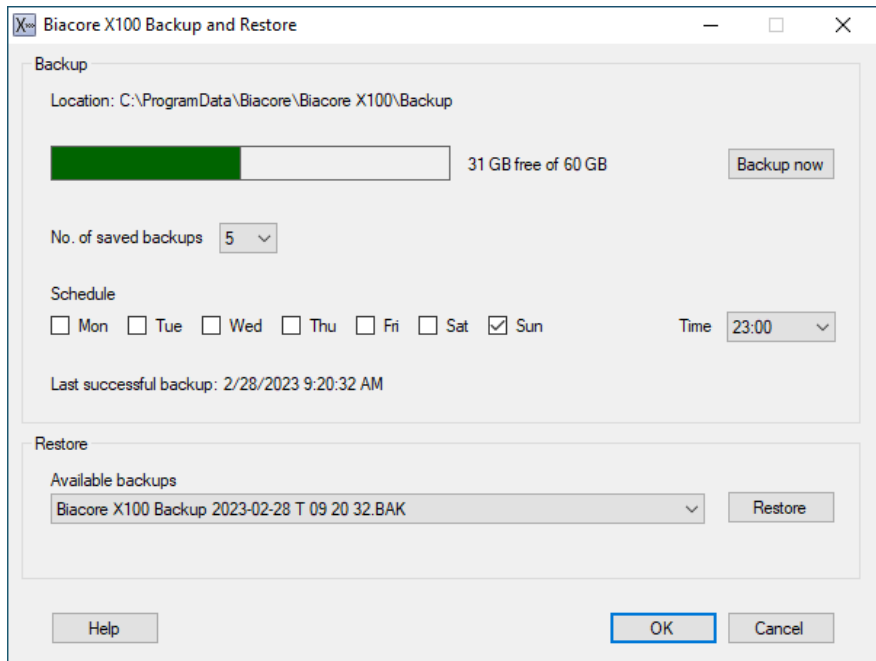
F.4 Database backup

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 Sundays. Five backups are saved by default. When the set number of backups is reached, the oldest backup is deleted for each new backup saved.

The backup files are saved locally. It is the responsibility of the users to follow their local backup policies and save the files off-site if required.

Users with local administrator rights can change the backup settings on the computer where the database is stored, using the Biacore X100 Backup and Restore application. Click **OK** to apply the new settings. The settings are also automatically applied when **Backup now** is clicked.

Note: *The folder in which the backup files is saved is hidden by default. Hidden items can be made visible from settings in Windows Explorer.*



F.5 Restoring a database

Users with sysadmin rights in the Biacore X100 database, such as **SQLServerX100DatabaseAdministrators**, can restore a saved backup from the Biacore X100 Backup and Restore application. Select which backup to restore and click **Restore**. It is not possible to specify what data to restore within a backup.

Note: *Restoring a database overwrites all current database contents.*

Note: *It is important to close all instances of Biacore X100 Control Software and Biacore X100 Evaluation Software before a restore to prevent data damage.*

F.6 Database connections

To view Biacore X100 data you need to have access to the database. If you access the local database on the system controller from another computer via a network, you have to define the database connection.

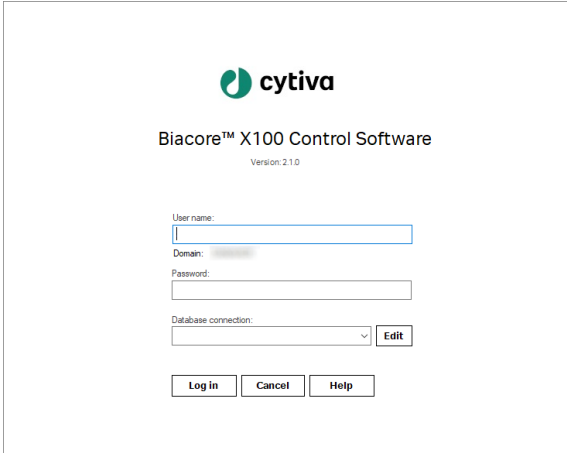
In this section

Section	See page
F.6.1 Selecting a database at login	185
F.6.2 Managing databases	186

F.6.1 Selecting a database at login

Step	Action
------	--------

1	Start Biacore X100 Control Software or Biacore X100 Evaluation Software.
---	--



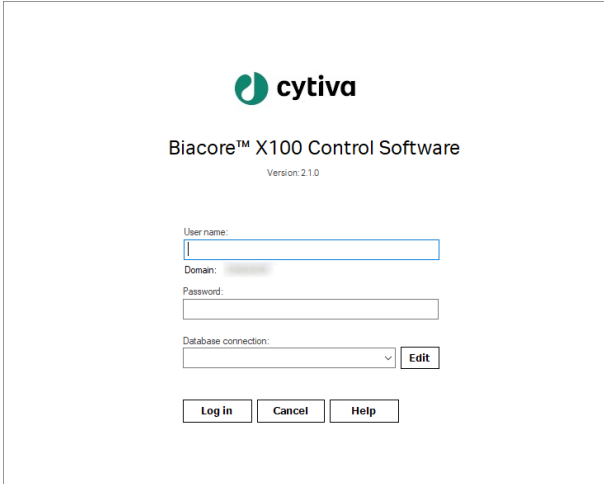
The screenshot shows the login interface for Biacore X100 Control Software. It includes a logo, version information, and fields for user credentials and database selection.

2	In the login dialog, select the database from the Database connection list.
---	--

F.6.2 Managing databases

Step	Action
------	--------

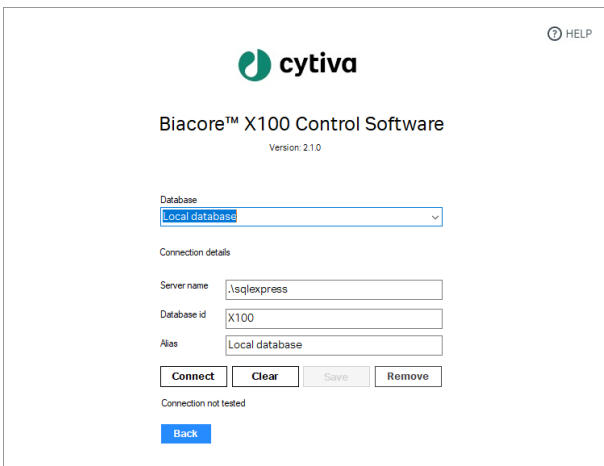
- 1 In the login dialog on the system controller, click **Edit**.



- 2 Under **Connection details**, enter correct server name and database id, and an alias for the database connection to be used in the **Database** drop-down list.

Note:

Make sure to use an alias that does not already exist in the drop-down list, otherwise the existing database connection with that alias is edited.



- 3 (Optional) Test the database connection:
a. Click **Connect**.

Step	Action
	b. In the pop-up, enter your user credentials to test the connection.
	c. Click Connect to test the database connection and close the pop-up.
4	Click Save to save the database connection.
5	Click Close to return to the login dialog.

F.7 Re-installing the Biacore X100 software

The Biacore X100 database and software are set up on the system controller by the service representative at the time of installation. In the event that reinstallation is required, installation instructions may be found on the Cytiva web site at [cytiva.com/biacore](https://www.cytiva.com/biacore). Instructions are also provided for installing additional copies of the software on other computers that access the database on the system controller.

Index

Numerics

- 1:1 binding, 155
- 4-parameter equation, 169

A

- absolute response, 16
- active concentration, 102
- advanced filter, 63
- affinity assays, 37
- ambient temperature, 124
- analysis temperature, 47, 98
- analyte, 16, 163
- append Result, 81
- Append Result, 81
- applying solvent correction, 176
- assay conditions capture, 73
- assay conditions sample, 73
- assay development steps, 66
- assay duration, 34
- assay performance, 95
- assay principles, 15
- assay step, 68
- assay steps, 65
- assay wizards, 65, 73
- assay workflow, 61, 65
- attaching ligands to the sensor surface, 30
- avidity, 156

B

- bar chart, 88
- basic operation, 35
- Biacore templates, 74, 99
- Biacore terminology, 16
- Biacore X100 administrator, 61
- Biacore X100 system, 14
- binding analysis, 26, 73
- binding capacity, 163
- bivalent analyte, 156
- buffer bottle, 19, 39, 42
- buffer recommendations, 33
- bulk contribution, 173
- bulk refractive index contribution, 164

C

- calculated parameters, 165
- calibrants, 102
- calibrated concentration analysis, 27, 91, 102
- calibration curve, 27
- calibration-free concentration analysis, 27, 91, 103, 170
- capture conditions, 66
- capturing ligands, 30
- capturing molecule, 16, 66
- carboxymethyl dextran matrix, 31
- carry-over, 115
- CFCA, 27, 91, 103, 170
- changing buffer, 42
- checking for leaks, 109
- chemical resistance, 135
- chi-squared, 148
- choosing ligand attachment approach, 32
- cleaning, 110
 - instrument, 110
- cleaning the needle, 109
- competing analytes, 157
- complex, 163
- concentration analysis, 27, 73, 101
- concentration analysis evaluation, 91
- conformational change, 159
- connecting to the PC, 128
- constants, 149
- contamination with biohazards, 107
- correlated parameters, 152
- covalent immobilization, 30
- creating a workflow, 66, 68
- creating fitting models, 161
- curve fitting, 147
- custom assay wizard, 73, 99
- custom immobilization methods, 105
- custom report points, 94

D

- damaged needle, 115

data quality check, 95
 database, 179
 database backup, 182
 database connection, 184
 database contents, 61
 database items, 61
 database management, 186
 database restore, 183
 degasser, 42, 97
 Desorb, 108, 115
 detecting molecule, 140
 detection system, 137, 141
 dextran matrix, 31
 diffusion coefficient, 92, 103, 154, 170
 diffusion properties, 27
 dimethyl sulfoxide, 28, 173
 DMSO, 28, 93, 104, 173
 drift, 164

E

editing a workflow, 70
 emergency stop, 12
 evaluating multiple runs, 81
 evaluating results, 74
 evaluation, 61
 evaluation explorer, 79, 82
 evaluation items, 83
 evaluation requirements, 94
 evaluation software, 78
 general features, 78
 evanescent wave, 138
 excluded volume effect, 173
 excluding data from evaluation, 95
 exporting database items, 63
 extrapolating solvent correction curves, 176

F

filter tabs, 61
 find capture conditions, 66
 find immobilization pH, 66
 find regeneration conditions, 67
 find sample conditions, 66
 finding database items, 63
 finishing a run, 53
 fitting models, 106
 flow cells, 44, 144
 flow rate, 170

G

global parameters, 149

H

HBS-EP+, 33
 heterogeneous analyte, 157
 heterogeneous ligand, 158
 hiding the evaluation explorer, 82

I

IFC, 143
 immobilization, 16
 immobilization pH, 66
 immobilization pH scouting, 73
 immobilizing ligand, 30, 66
 Important user information, 6
 importing database items, 63
 initial binding rate, 92
 initial values, 165
 Inserting a sensor chip, 45
 installation, 120
 instrument maintenance, 107
 interaction models, 4, 161–165
 interaction monitoring, 14
 internet connection, 60

K

keyword requirements, 94
 keyword table, 94
 kinetics, 153
 predefined models, 153
 kinetics assays, 25
 kinetics/affinity analysis, 73
 kinetics/affinity evaluation, 89
 km, 170

L

leaks, 109
 learning to use Biacore, 18
 leaving the instrument, 53
 ligand, 16, 163
 ligand attachment, 31
 ligand capture, 30
 ligand immobilization, 30, 66
 linear fit, 169
 lipids, 31
 liquid flow problems, 117

loading the sample rack, 51
 local parameters, 149
 log in, 3, 57, 58
 low molecular weight analytes, 21,
 28, 57, 93, 104

M

mains inlet, 127
 mains power, 4, 120, 123
 maintenance, 107
 overview, 107
 Maintenance Kit, 107
 manual run, 75
 mass transfer, 163
 mass transfer coefficient, 154, 170
 mass transfer constant, 152, 154
 mass transport limitation, 170
 membrane preparations, 31
 monitoring interactions, 14
 moving the instrument, 4, 120,
 125–128
 multi-cycle kinetics, 25

N

needle damage, 115, 116
 needle replacement, 115
 Notes and tips, 6

O

opening data for evaluation, 80
 opening database items, 63
 organic solvents, 21, 57, 93, 104

P

parameter significance, 152
 password, 41
 peristaltic pump, 40
 plasmons, 138
 plot window, 87
 Plus Package, 14, 42, 47, 74, 91, 96
 power lamp, 38
 power requirements, 123
 predefined models, 4, 146,
 153–160
 predefined plots, 80, 87
 preparing buffers, 42
 preparing samples, 50
 presenting results, 85

preventive maintenance, 110
 principles of Biacore assays, 15
 pump calibration, 108, 116
 pump compartment, 145

Q

QC ratio, 170, 171
 acceptance, 171
 quick filter, 62

R

rack locked indicator, 51
 ranking, 87
 rate equations, 165
 rating information, 11
 reactants, 162
 reagent rack, 133
 recombinant proteins, 32
 reference subtraction, 173
 refractive index, 138
 refractive index change, 14
 regeneration, 15, 16
 regeneration conditions, 68
 regeneration scouting, 73
 regression coefficient, 151
 regular maintenance operations,
 108
 relative response, 17
 reliability of KD values, 160
 report point range, 176
 report point table, 80, 94
 report points, 14, 17
 requirements for assay evalua-
 tion, 94
 resonance unit, 16
 response, 16
 response correction factor, 157
 restart Biacore X100, 12
 restoring a database, 183
 result items, 61
 results presentation, 85
 right-click menus, 3, 78, 84
 Run lamp, 38
 running buffer, 16, 33, 134

S

safety, 8, 11
 information, 8
 label, 11

- Safety, 8
 - instructions, 8
 - Safety notices, 8
 - Safety precautions, 8
 - introduction, 8
 - salt residues, 115
 - sample compartment, 19
 - sample conditions, 66
 - sample preparation, 33, 50
 - sample vials, 51
 - Sanitize, 108
 - SE-value, 152
 - sensor chip, 14, 31, 43, 142
 - Sensor chip, 45
 - inserting, 45
 - Sensor Chip lamp, 38
 - sensor chip port, 19
 - sensor chip types, 31
 - sensorgram, 14, 17
 - serial communication, 128
 - shutdown, 112
 - significance of fitted parameters, 152
 - single-cycle kinetics, 25
 - software overview, 20
 - software problems, 118
 - solutions, 135
 - parts in contact with, 135
 - solvent correction, 21, 28, 57, 81, 93, 104, 172, 174, 176
 - applying, 176
 - extrapolation, 176
 - principle, 174
 - solvent correction principle, 174
 - space requirements, 122
 - specifications, 129
 - SPR, 138
 - SQL server, 179
 - standard error, 152
 - standby mode, 111
 - starting evaluation software, 77
 - starting the instrument, 36
 - starting the system, 36
 - status indicators, 19
 - steady state, 89
 - steady state affinity, 160, 166
 - steps in a workflow, 66
 - stop run, 12
 - superclean, 118
 - support, 14, 22
 - Support Navigator, 60, 79
 - surface plasmon resonance, 138
 - syringe pump, 115
 - System Check, 108, 116
 - system event log, 108
- ## T
- T-value, 152
 - tagged molecules, 32
 - technical specifications, 129
 - temperature, 38, 47
 - lamp, 38
 - temperature control, 47, 98
 - terminology, 16
 - time to reach steady state, 89
 - Tools, 116
 - system check and pump, 116
 - calibration, 116
 - troubleshooting, 114
 - two-state reaction, 159
 - Typographical conventions, 6
- ## U
- U-value, 152
 - uniqueness, 152
 - unstable temperature, 118
 - user administration, 181
 - user folders, 67
 - user name, 41
 - user-defined fitting models, 106, 161
- ## V
- vial caps, 115
- ## W
- waste bottle, 19, 39, 41
 - water vial, 51
 - web-based support, 60
 - when to use solvent correction, 175
 - wizard templates, 61, 74
 - wizards, 65, 73
 - workflow, 61, 65
 - workflow settings, 65

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