Biacore® X

The versatile high sensitivity system

- Work with high sensitivity
- Study binding in non-aqueous and aqueous samples
- Reduce sample consumption
- Perform the most advanced kinetic evaluation
- Use proven technology

BIACORE® X

Introduction

Biacore® X is a high sensitivity, semi-automated system for label free studies of biomolecular binding, in samples ranging from small molecules to crude extracts, lipid vesicles, viruses, bacteria and eucaryotic cells. Biacore X answers questions about the speed, strength and specificity of binding and determines the active concentrations of components. Results are evaluated using the most advanced evaluation software available. Before and after analysis a simulation program can be used to perform dry runs, to test experimental conditions or to verify experimental data, saving time and materials.

Biacore X is ideal for rapid, single runs and provides accurate sensorgrams smoothly and easily. After loading the sample, the system controls every critical step from sample injection to real time display. Biacore X is designed for laboratory environments where many users handle different types of sample in small numbers.

Work with high sensitivity

Under standard experimental conditions Biacore X can detect up to 70000 RU*, with a relative working range of 100 RU. However these values have been surpassed under fully optimized conditions. This high sensitivity allows the study of samples ranging from small molecules to large surface complexes.

**RU: Biomolecular binding events at a sensor surface cause changes in an SPR signal which are expressed in resonance units, RU (one RU is equivalent to one picogram per square millimetre of sensor surface).*

BIACORE

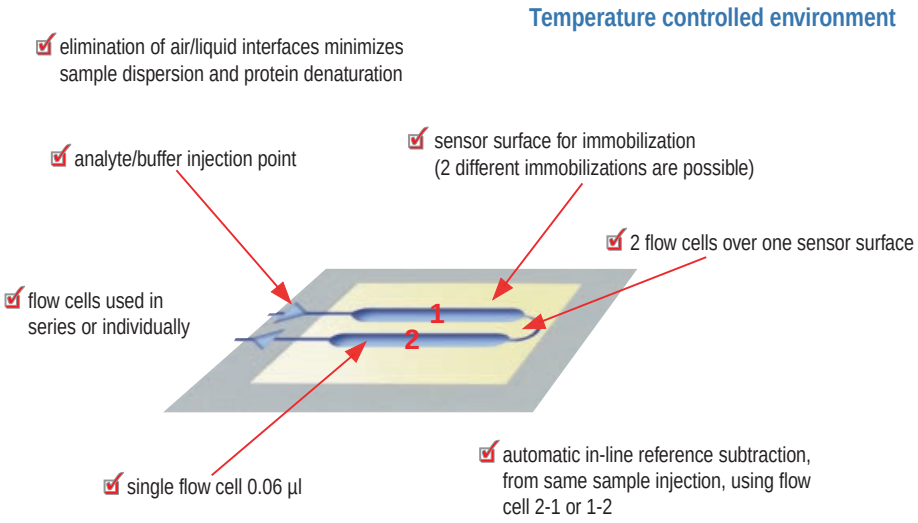


Fig. 1. Microfluidic system of Biacore X housed in a temperature controlled environment.

Measurements of kinetic and affinity parameters are always dependent on the experimental conditions as well as the molecular weights of the binding partners. The microfluidic pathway of Biacore X, shown in Figure 1, incorporates a single sensor surface overlaid by two flow cells, each with a volume of 0.06 µl. One flow cell can be used as a true reference during a single sample injection. Automatic in-line reference subtraction maximizes the resolution and information from a single run. The reduction of background noise will resolve signals which may not otherwise be observed. Kinetic and concentration measurements can also be improved by studying two surface ligand concentrations in the same run.

The controlled environment of the microfluidic flow path of Biacore X, together with the automatic reference subtraction, allow the measurement of small molecules and the measurement of weak binding, as shown in Figure 2. This example shows the binding of maltose (MW 360 Da) to an immobilized antibody with an apparent affinity of 6 x 10^{-5} M^{-1} .

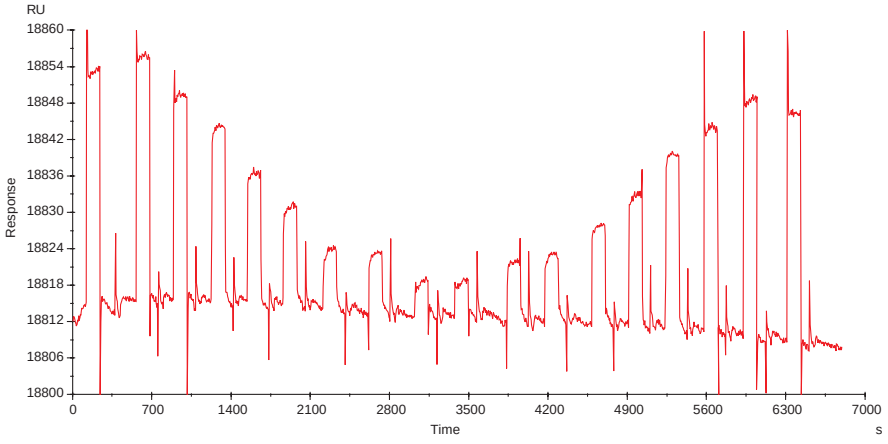


Fig.2. In line reference subtracted data: sequential injections of a maltose (MW 360 Da) concentration series. Results show an injection series beginning with decreasing concentration and followed by increasing concentration.

Courtesy of Sten Ohlsson, Kalmar Högskola, Sweden.

Study binding in non-aqueous and aqueous samples

Biacore X is used for samples ranging from small molecules to crude extracts, lipid vesicles, viruses, bacteria and eucaryotic cells. It may be necessary, for solubility or stability reasons, to work with specific additives, such as organic solvents or high salt concentrations.

The detection system of Biacore X allows sample measurements over a broad dynamic range to enable the performance of binding studies in a range of solutions, as shown in Figure 3.

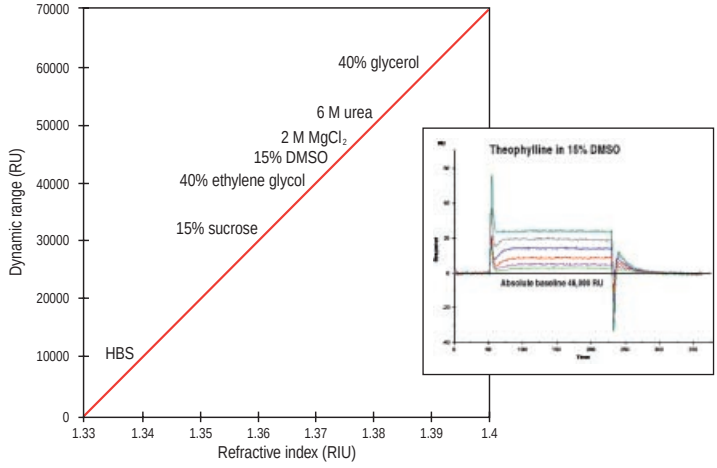


Fig.3. Binding can be studied in a wide range of solutions.

Automatic in-line reference subtraction easily eliminates major refractive index effects. Figure 3 also shows an example in which the binding of theophylline (MW 180 Da) to an immobilized antibody is confidently measured despite a high back-ground response generated by 15% DMSO.

Reduce sample consumption

Samples used in binding studies are often valuable and available only in small quantities. Biacore X is designed to reduce sample consumption whenever possible.

Sample injections from as little as 5 µl are required to monitor binding in both flow cells, each flow cell having a volume of 0.06 µl. By immobilizing a different ligand in each flow cell, the same sample can be used to study binding on two different surfaces, as was done to produce the results shown in Figure 4.

Sample flow-through material can be collected during injection and surface-bound sample can be recovered for complementary analysis. The highest quality sensor surfaces minimize non-specific binding.

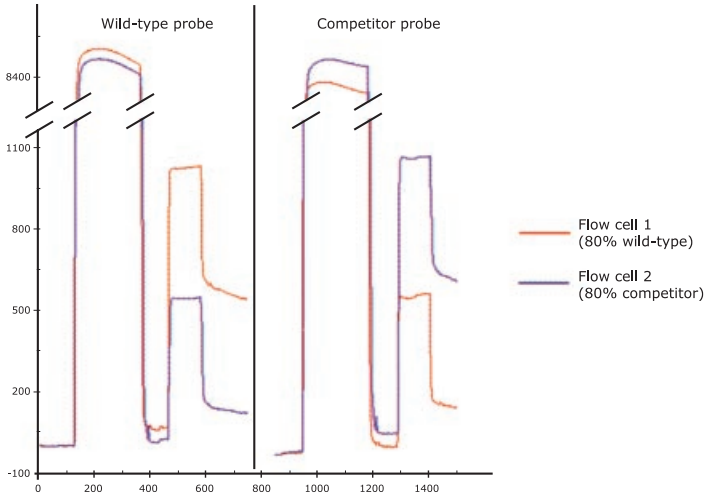


Fig.4. With different proportions of wild-type and competitor DNA sequences immobilized in the two flow cells, PCR products are selectively detected. Response in each flow cell reflects the relative amounts of immobilized DNA species.

Perform the most advanced kinetic evaluation

Biacore X includes options for the most advanced kinetic evaluation, and performs affinity or concentration calculations. The global fit functions in BIAevaluation further extend the range for quantitative kinetic analyses.

Evaluation Wizards, tool tips and pre-programmed models reduce the need for mathematical expertise and accelerate the evaluation of even the most complex interactions.

For more detailed information on BIAevaluation please ask for Product Information Sheet BR-9000-85.

Use proven technology

Biacore X has been used successfully for many different applications and a regularly updated list of publications is available on http://www.biacore.com. Two examples using Biacore X are shown below:

Binding of thrombin-activated coagulation factor VIII (fVIIIa) to phospholipid surfaces is required for assembly of one of the multi-protein complexes involved in the coagulation cascade. Using Biacore X, Saenko et al have shown that the affinity of fVIII for phospholipid monolayers increases upon thrombin activation (Figure 5).

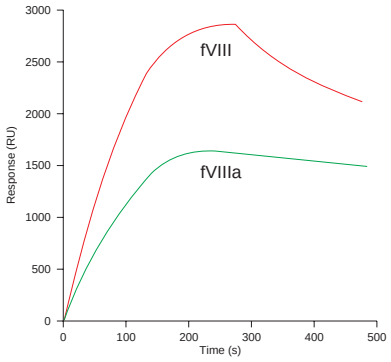


Fig. 5. Binding of 10 nM each of factor VIII (fVIII) and thrombin-activated factor VIII (fVIIIa) to a phosphatidyl serine/phosphatidyl choline (25/75) monolayer on the surface of Sensor Chip HPA in the presence of 200 nM A2 subunit. FVIIIa has a ten-fold higher affinity than that of fVIII.

Traditionally the assembly of multi-protein complexes of coagulation proteins on lipid monolayers has been performed using stopped flow techniques, but Saenko reports several advantages of using a Biacore X, including technical simplicity, no requirement for phospholipid labelling, less sample used and a phospholipid surface that can be re-used to allow straightforward assessment of accuracy and reproducibility.

Further details: BIAjournal, Vol. 5, No. 2, p. 29 and J. Biol. Chem., 273, 27918-27926, 1998.

DNA-dependent protein kinase (DNA-PK) is the only eukaryotic protein kinase known to be specifically activated by double-stranded DNA (dsDNA) termini. This explains the importance of DNA-PK in the repair of dsDNA. West et al studied the binding of DNA-PK to DNA termini of various lengths in the presence or absence of Ku (a molecule known to be involved in the activation of DNA-PK). Biacore X was used to monitor the build up of multi-molecular complexes between Ku, DNA-PK and dsDNA and to analyze the kinetic events. The authors concluded that for dsDNA smaller than 26-bp, DNA-PK is inactive as a kinase despite being bound to Ku:DNA.

However for dsDNA above 26-bp the presence of Ku stimulates DNA-PK activity by increasing the stability of the DNA-PK-Ku-DNA complex. Figure 6 shows examples of sensorgrams from this work.

Further details: Mol. and Cell. Biol., 18, 5908-5920, 1998.

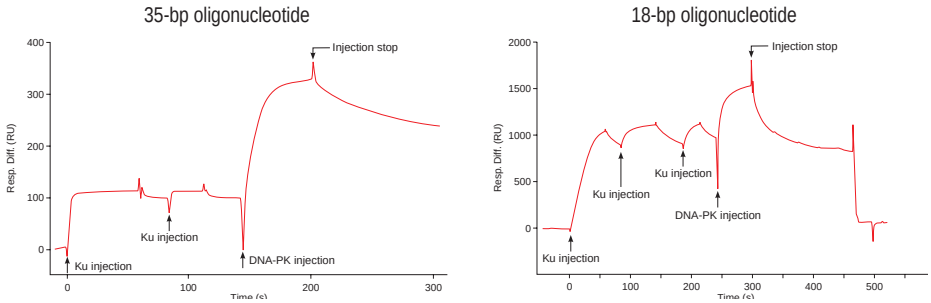


Fig. 6. Sequential injections of Ku (28.5 nM) and DNA-PK (2.4 nM) over Sensor Chip SA loaded with a 35-bp oligonucleotide (a) or an 18-bp oligonucleotide (b) show the binding characteristics of DNA-PK with the Ku:35 bp DNA fragment and Ku:18-bp DNA fragment respectively.

Biacore AB would like to thank the authors of these applications for their kind permission to reproduce this work.

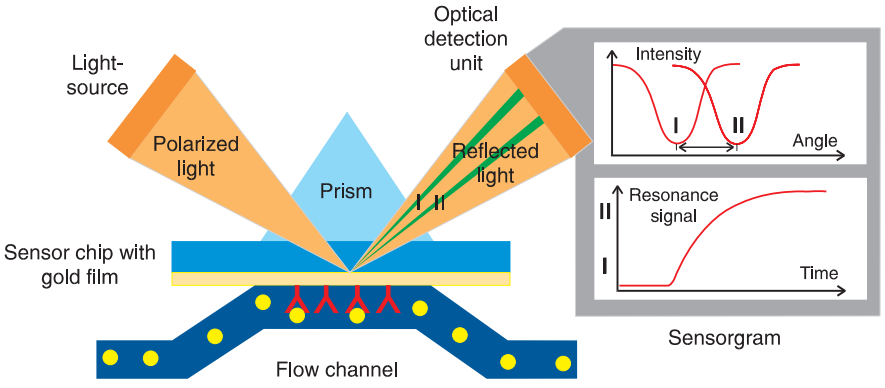
For the best results in biomolecular binding studies

1. **Control of every critical step**
2. **Optimized monitoring**
3. **Advanced evaluation**

1. Control

- Real time display shows binding events, for assessment of kinetics and immediate feedback
- Step-by-step method development for specialized applications
- System control ensures that sample and reagents are delivered to the sensor surface in a precise, pulse free flow to give reproducible injections

Biacore systems use the phenomenon of surface plasmon resonance (SPR) to monitor biomolecular binding events in real time without the use of labels. SPR occurs when surface plasmon waves are excited at a metal/liquid interface *(the sensor surface)*.



Biacore's integrated system

Light is directed at, and reflected from, the side of the surface not in contact with sample. SPR causes a reduction in the reflected light intensity at a specific combination of angle and wavelength *(generating a refractive index dependent SPR signal)*.

Molecules binding to the sensor surface cause changes in the refractive index close to the surface which are detected as changes in the SPR signal (expressed in resonance units, RU where one RU is equivalent to one picogram per square millimeter on the sensor surface). In general, the refractive index change for a given change of mass concentration at the surface layer, is practically the same for all proteins and peptides, and is similar for glycoproteins, lipids and nucleic acids.

Biomolecular binding events cause further changes in the refractive index and the SPR signal. Biacore X precisely controls every critical step to detect the small changes between a baseline signal and the signal generated when a second or third molecule interacts.

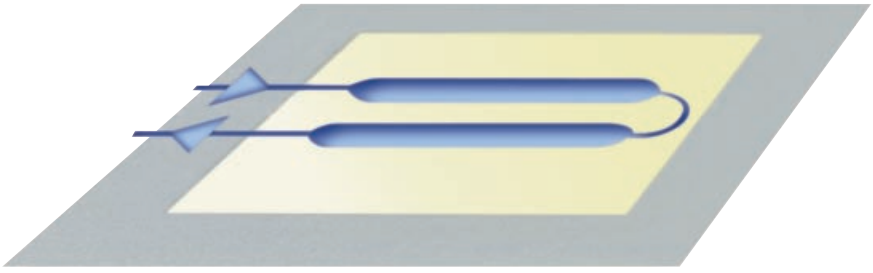
2. Monitor

- Temperature controlled detection environment with a high data acquisition rate replace result in a high signal to noise ratio
- Detection system allows studies in non-aqueous and aqueous samples, with target molecules in native form

2a. Microfluidic system

- Flow cells designed to use as little as 5µl of sample
- Elimination of air/liquid interfaces minimizes protein denaturation
- Fast buffer/sample exchange rates minimize sample dispersion
- Continuous injection maintains sample concentration ensuring accurate rate constant determination
- Two flow cells allow immobilization of 2 different molecules
- Dual-channel detection and serial flow allow monitoring of 2 different interactions simultaneously
- In line reference subtraction from the same sample injection ensures perfect controls
- Sample recovery for complementary analysis

Biacore's unique integrated fluidics design



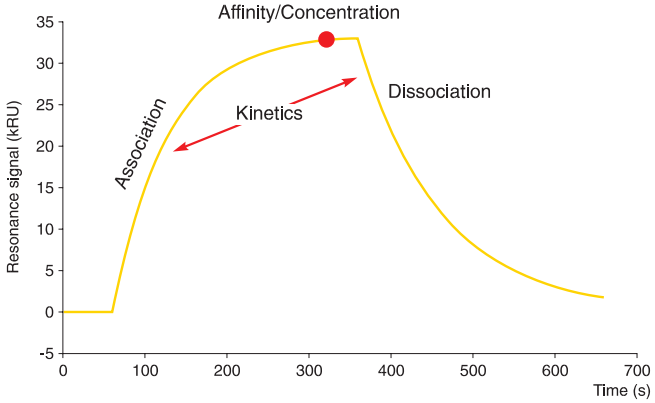
2b. Sensor surfaces

- A wide selection of sensor surfaces to maximize application flexibility
- Highest quality sensor surfaces to minimize non-specific binding and protein denaturation
- Stable surface chemistry ensures low drift and tolerance to very harsh conditions

Extensive range of sensor chip surfaces

3. Evaluate

- Global fit and numerical integration for simultaneous analysis of several data sets and evaluation of kinetic constants
- Determination of affinity constants for surface and solution interactions
- Determination of active concentration from binding level or binding rate data
- Theoretical investigations by simulation from models and kinetic parameters



The sensorgram output of Biacore systems

System Specifications

Sample handling:	Manual sample loading/automated injection
Molecular weight detection:	>180 Da
Detection performance *Myoglobin*:*	10 pM
Flow rate range:	1 - 100 µl/min, through flow cell, steps of 1 µl
Required sample volume:	injection volume +20 µl
Refractive index range:	1.33 - 1.40
Analysis temperature:	4 - 40°C (max 10°C below ambient)
Number of flow cells:	2 (used individually or in series)
In-line reference subtraction:	yes
Dimensions (L x W x H):	584 x 462 x 317 mm
Electric voltage:	100 - 120 V; 220 - 240 V
Power consumption:	max 580 VA
Net Weight:	25 kg/55 lbs

** Detection limits achieved with myoglobin 17 000 Da, measured with an antibody sandwich assay*

Ordering Information

Biacore X is delivered with processing unit, system controller, Biacore X control software, BIAevaluation, BIAsimulation and Windows®2000 operating system. Please contact your local Biacore representative for more information.

Related Products

BIAevaluation Software Kit	BR-1002-16
BIAsimulation Software Kit	BR-1002-30

Related Literature

BIAevaluation Product Information Sheet	BR-9000-85

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