

Biacore[®]3000



The high performance research system

- Work with high sensitivity
 - direct detection of small molecules at < 1 nM concentration
 - increased resolution for kinetic analysis
 - measurement of weak affinities
- Recover bound analyte in high concentration
- Deliver recovered analyte to vial or direct to MALDI target
- Shorten analysis times
- Minimize sample consumption
- Study binding in non-aqueous and aqueous samples
- Use Biacore Wizards to simplify and accelerate analysis
- Develop specialized applications efficiently
- Perform the most advanced kinetic evaluation

Introduction

Biacore 3000 is the highest performance research system available for label free studies of biomolecular binding. Samples ranging from small molecules to crude extracts, lipid vesicles, viruses, bacteria and eucaryotic cells can be studied. Biacore 3000 answers questions about the *speed*, *strength* and *specificity* of binding and determines the *active concentrations* of components. Technical innovations meet the highest demands for sensitivity, efficiency and flexibility.

With new wizard driven and automated recovery functions including the novel Surface Prep unit for increased capacity recovery, Biacore 3000 is an ideal tool for functional proteomics. Increased integration with mass spectrometry characterization is provided by the enhanced recovery and ability to directly deliver sample to a MALDI target as well as delivery of digestion enzymes and MALDI matrix, all within the autosampler of the system.

The knowledge and experience of Biacore has been incorporated into Wizards which guide the user without effort through preparation, experimentation and evaluation. Biacore Wizards provide a trend analysis and preliminary results at the end of runs. *Conditional IF/THEN statements* ensure that Biacore 3000 responds correctly to changes in run conditions.

The most advanced software available enables detailed evaluation of results. Biacore 3000 is designed for individual sample characterization where the highest resolution in kinetic analysis is essential (see Figure 1) and for automation of multi-sample analyses (see Figure 2).



BIACORE

Superior performance for kinetic analysis

Fig. 1a. Sample characterization

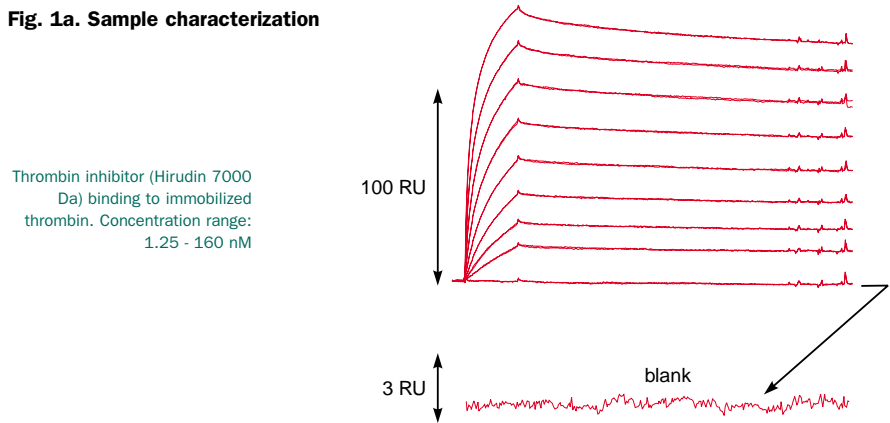
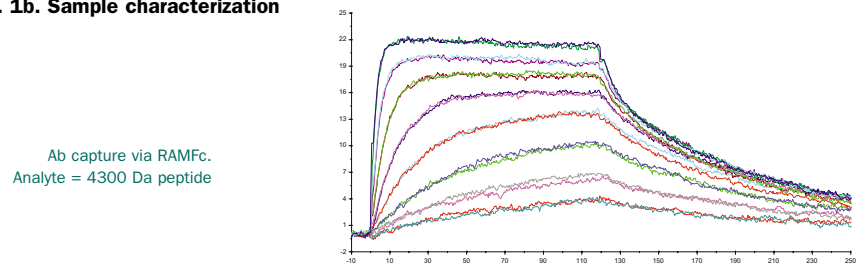


Fig. 1b. Sample characterization

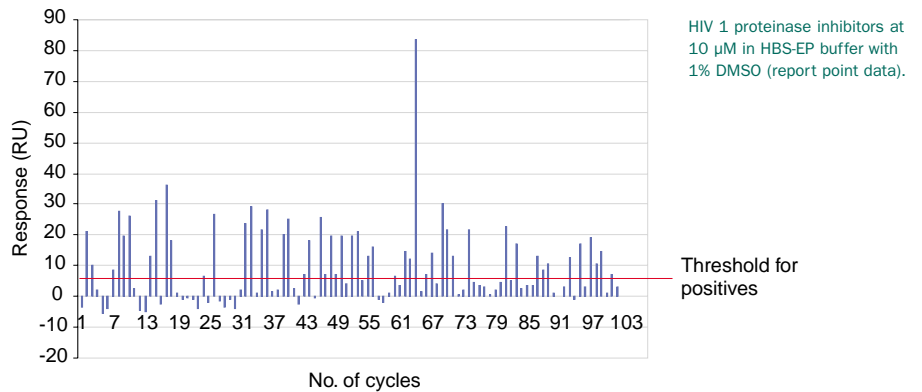


Compound	MW
A001	539
A005	539
A006	574
A009	570
A010	655
A011	562
A012	538
A021	635
A025	659
A026	674
antipyrine	188
atenolol	266
B252	777
B268	614
B272	356
B274	370
B277	519
B295	603
B347	614
B369	652
B383	650
B384	719
B385	599
B400	667
B411	562
B412	857
cholic acid	409
desipramine	302
d-glucose	180
dl-propranolol	296
furosemide	330
hydrochlorothiazide	298
iopanoic acid	571
iophenoxic acid	572
metoprolol	649
naproxen	230
nelfinavir	664
phenylbutazone	308
piroxicam	331
quinine	324
ritonavir	721
salbutamol	239
sulphadimetazine	278
terbutaline	274
tolterodine	325
urea	60
warfarin	308
verapam II	491
vx478	506

Biacore 3000 offers superior performance for kinetic analysis. Increased resolution is achieved by the highest signal to noise ratio, a high data acquisition rate and automatic in-line reference subtraction.

Ideal for multi-sample analysis

Fig. 2. Multi-sample analysis



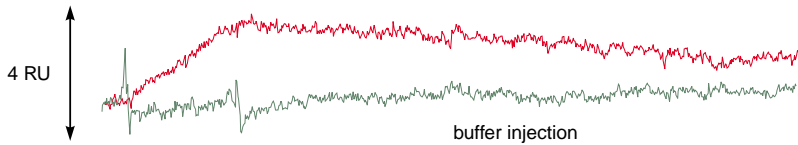
Biacore 3000 is ideal for multi-sample analysis. In this example the automatic in-line reference subtraction and optimized wash routines ensured efficient screening of 49 low molecular weight (<1000 Da) compounds. Biacore 3000 ran 102 cycles within 39 hours.

Work with the high sensitivity

Biacore 3000 can detect up to 70000 RU*, yet has a relative working range of as little as 10 RU. The ability to monitor biomolecular binding at this highest sensitivity ensures confidence in both the detection of binding events and in the interpretation of the related kinetic data.

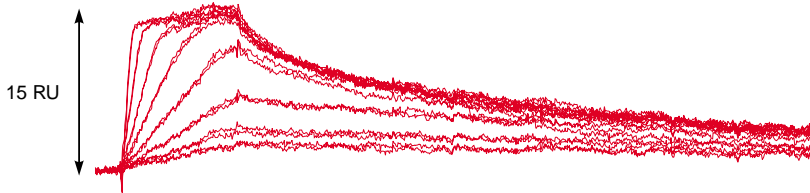
Figure 3 shows examples of the high sensitivity achieved with Biacore 3000.

Fig. 3a. Direct detection of small molecules at < 1 nM concentration



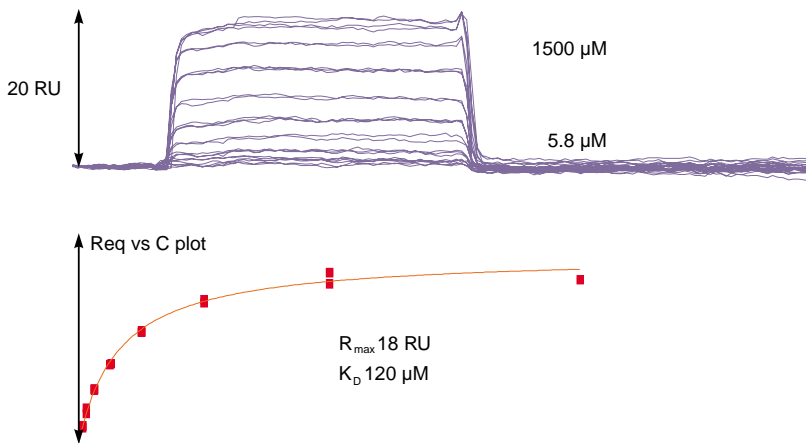
Thrombin inhibitor (Melagatran 420 Da) binding to immobilized thrombin. Concentration: 0.78 nM

Fig. 3b. Increased resolution for kinetic analysis



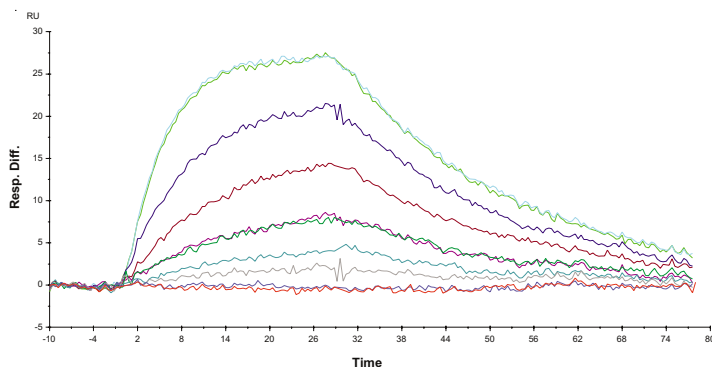
Thrombin inhibitor (Melagatran 420 Da) binding to immobilized thrombin. Concentration range: 0.39 - 50 nM (duplicate injections). Lower concentrations can be tested by increasing the sample injection or contact time.

Fig. 3c. Measurement of weak affinity binding events



Maltose (360 Da) binding to immobilized anti-maltose antibody. Concentration range: 5.8 - 1500 μM (duplicate injection of each concentration).

Fig. 3d. Furosemide (0-10 μM) binding to carbonic anhydrase

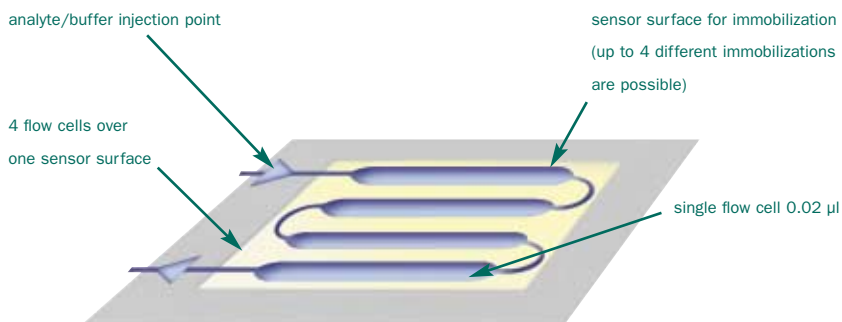


* RU: Biomolecular binding events at a sensor surface cause changes in an SPR signal which are expressed in resonance units, RU (one RU is equivalent to one picogram of protein per square millimetre on the sensor surface).

Measurements of kinetic and affinity parameters are always dependent on the experimental conditions as well as the molecular weights of the binding partners. Technical innovations in the design of the microfluidic pathway and detection system of Biacore 3000 ensure precisely controlled experimental conditions, as shown in Figure 4. Twice the signal can be generated for the same sample injection time compared to other Biacore systems. The microfluidic pathway of Biacore 3000 incorporates a single sensor surface overlaid by four flow cells, each with a volume of 0.02 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.

Fig. 4. Microfluidic system of Biacore 3000 housed in a temperature controlled environment

- elimination of air/liquid interfaces minimizes sample dispersion and protein denaturation



- flow cells used in series or individually
- automatic in-line reference subtraction, from same sample injection, using flow cell 2-1, 3-1, 4-1, 4-3

Four sensorgrams (one for each flow cell) and three reference subtracted curves can be displayed per single run. Flow cells 1-2 and 3-4 can be used as separate pairs for increased efficiency.

All parameters which have been optimized in Biacore 3000, to achieve such superior performance in binding studies, are shown on the inside back page of this document.

Shorten analysis times

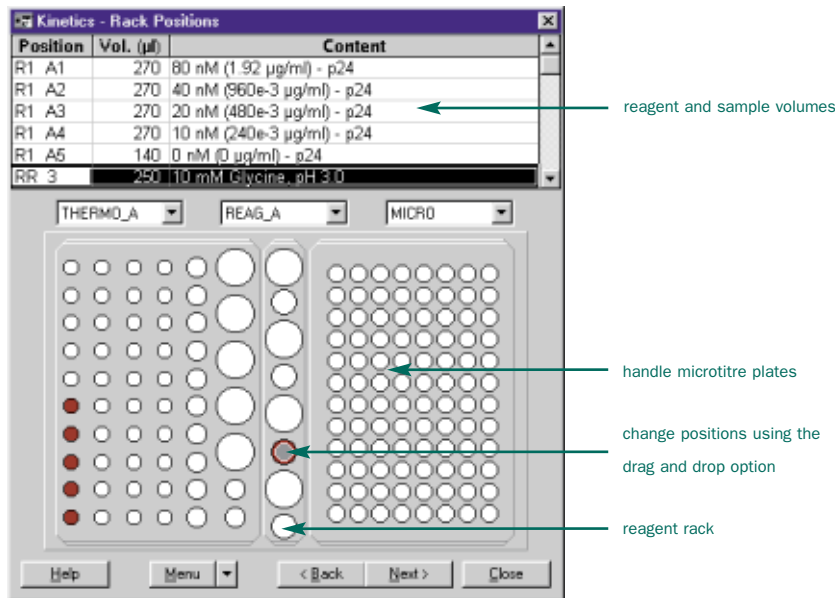
System use may vary from a single sample run to unattended multi-sample runs over many hours. Biacore 3000 selects samples automatically from microplates or vials.

Technical innovations in the microfluidic pathway shorten individual analysis times by halving the time previously needed to reach a satisfactory signal. Together with optimized wash routines between the runs this will, for example, save an estimated *five hours* in a 50 cycle run where each sample injection time is 5 minutes. More time can be saved by using *conditional IF/THEN statements* to enable Biacore 3000 to respond to pre-defined conditions, eliminating any unnecessary steps during a procedure (see section ‘Develop specialized applications efficiently’ for more details).

Automated in-line reference subtraction enables:

- fast visualization of the true response signal
- maximized resolution and information from a single run
- simplified evaluation processes

Fig. 5. Assay setup wizard



Biacore Wizards calculate the reagent volumes required for a programmed procedure.

Fig. 6. Large reagent vial rack for unattended runs



A large reagent vial rack filled with the appropriate volumes ensures that the system can be left unattended to run all samples without the need to check on reagent levels.

Minimize sample consumption

Samples used in binding studies are often valuable and available only in small quantities. Biacore 3000 requires sample injections from as little as 5 µl to monitor binding in all four flow cells. Each flow cell has a volume of only 0.02 µl.

Technical innovations in the design of the microfluidic pathway reduces the amount of sample required to achieve a satisfactory signal.

Biacore Wizards calculate minimum sample volumes required.

Enhanced recovery and integration with mass spectrometry

Surface-bound analyte can be recovered in high concentration in a defined volume of regeneration solution. Using the Analyte Recovery Wizard, MS-compatible buffer is injected into the system prior to sample injection. Directly after this injection, the IFC (including the flow cell area), is flushed briefly with the same buffer.

The autosampler and IFC (but not the flow cells) are further washed to prevent carry over prior to recovery of the bound analyte. The recovery is achieved by the passing of air segments over the flow cells for approximately 30 seconds before and after a 2 µl segment of recovery solution is incubated in the flow cells. After a user defined period, the 2 µl segment is drawn back into the autosampler and delivered to either a vial or a MALDI target.

Full information can be found in Technology Note 18 Analyte recovery in Biacore 3000: optimized functions for SPR-MS applications.

This enhanced recovery functionality and design modifications now offer increased integration with mass spectrometry and increased capacity recovery of samples of interest.

- Automated recovery functionality is wizard supported to simplify and improve efficiency of recovery.
- Analyte recovery commands are optimized to allow elution in a smaller volume and of higher purity.
- Assays optimized to recover captured materials bound on the chip surface in a state suitable for further analysis such as MALDI-MS.
- Washing procedures optimized to minimize carry over from chemical substances and other proteins.

The innovative Surface Prep Unit (Figure 7c) sitting in the autosampler of the Biacore unit provides increased analyte recovery.

- The surface area of 16mm² provided by the Flow Cell Carrier (FCC) type 2 is 3.3 times greater than the standard 4 flow cells of the IFC .
- Allows the user to optimize the surface to maximize bound analyte which is then automatically recovered and delivered as chosen in the wizard.

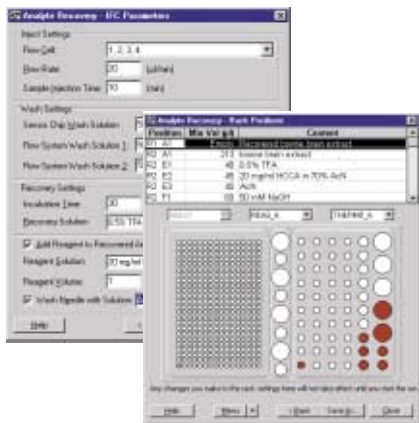


Fig. 7a. Analyte Recovery Wizard guides the user through the process of analyte recovery and delivery

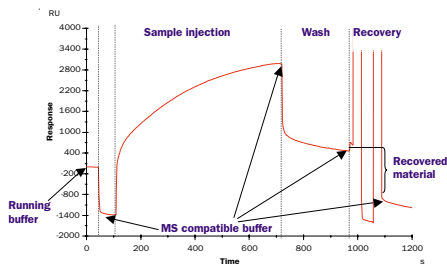


Fig. 7b. Sensorgram illustrating the main steps of an analyte recovery experiment run in Biacore 3000 with a sensor chip docked in the IFC. “Wash” here refers to a wash of the fluidic system and autosampler.

Integration with MS is achieved through direct deposition of recovered material to a MALDI target located in the autosampler area on a MALDI target holder (Figure 7d) (configured for Bruker MALDI targets).

- Wizard support for digestion of recovered protein after deposition into a vial or onto MALDI target
- Possibility to add matrix onto sample on MALDI target
- A simple MDL program to facilitate delivery of user defined digestion enzymes and MALDI matrix providing ultimate flexibility to suit mass spectrometrists's needs

External Immobilization

New external immobilization capability is also provided for immobilizations where reagents such as organic solvents are too harsh for the IFC.

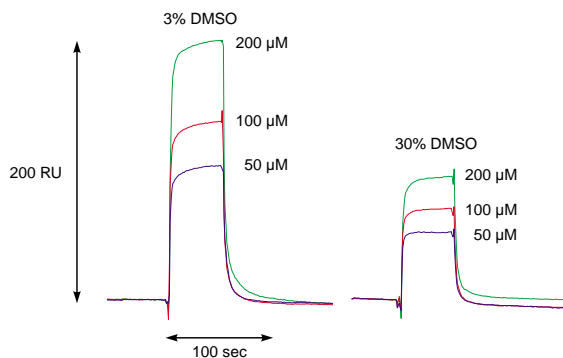
A second Flow Cell Carrier (type 1) is provided, creating 4 flow cells with the same surface area as those formed in the IFC. Once immobilization is complete, the chip can be inserted into the system for the assay to proceed.

Study binding in non-aqueous and aqueous samples

Biacore 3000 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. Technical innovations in the detection system of Biacore 3000 increase the dynamic range for sample measurements, enabling binding studies to be performed in a broad range of sample conditions (Figure 8a). Automatic in-line reference subtraction easily eliminates major refractive index effects.

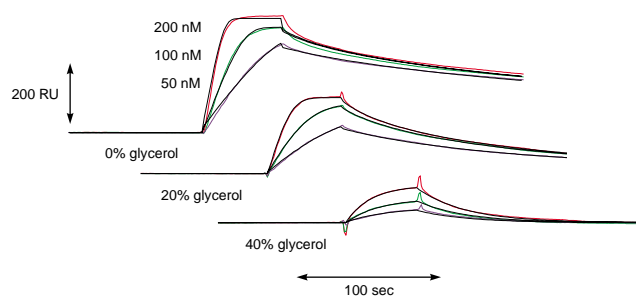
Examples include analyses performed in glycerol and DMSO environments (Figures 8b and 8c). The drop in signal level observed as the refractive index of a solution increases is due to a combination of the lower contrast between the refractive index of the sample and the running buffer and changes in the affinity characteristics. The broad range of sensor chip surfaces also enables selection of the optimal surface for the specific application.

Fig. 8b. DMSO environment. Iopanoic acid in 3% and 30% DMSO



Iopanoic acid (Mw 571 Da) binding to immobilized HSA. Iopanoic acid dissolved in HBS-EP buffer with 3% or 30% DMSO. Concentration range: 50 - 200 µM

Fig. 8c. Glycerol environment. Oligo, 9-mer, in 0, 20 and 40% Glycerol



Hybridization of 9-mer oligonucleotide (Mw 2778 Da) to an immobilized biotinylated 10-mer oligonucleotide. 9-mer oligonucleotide dissolved in HBS with 0.5 M NaCl and 0, 20 or 40% glycerol. Concentration range: 50 - 200 nM

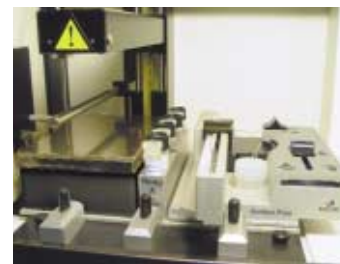


Fig. 7c. Surface Prep Unit and MALDI target holder in Biacore 3000 autosampler



Fig. 7d. Direct deposition to MALDI target

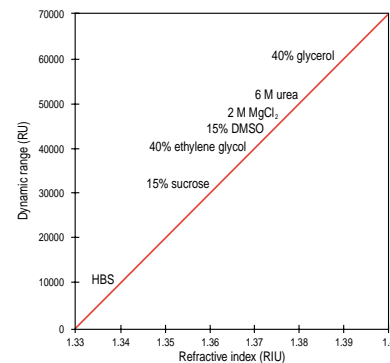


Fig. 8a. Samples measured in a broad range of solutions

Use Biacore Wizards to simplify and accelerate analysis

Biacore Wizards provide a fast and simple way of working with Biacore 3000, assisting and advising at every stage.

Surface Preparation Wizard

The Surface Preparation Wizard guides the user through the steps essential for preparation and testing of Sensor Chip CM5 and Sensor Chip SA. This Biacore Wizard:

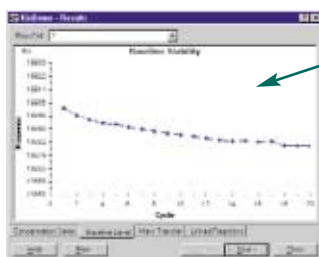
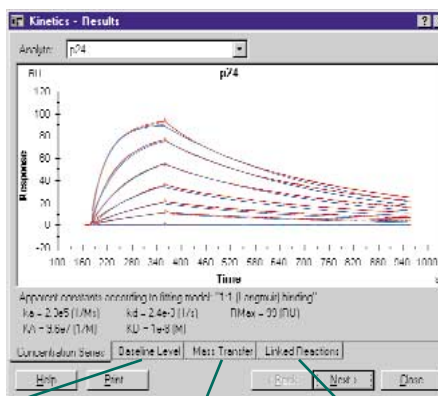
- scouts for correct immobilization pH
- helps to find suitable regeneration conditions
- guides through ligand immobilization
- checks surface performance for reproducibility and baseline stability



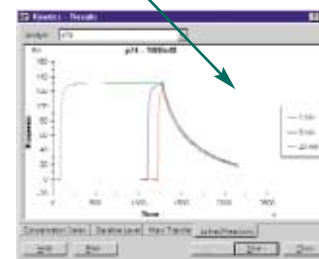
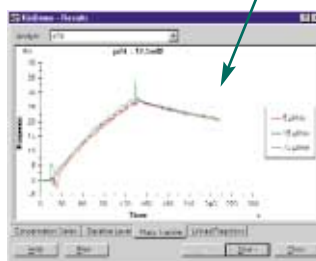
Fig. 9. Select a Biacore Wizard

Fig. 10. Kinetic Analysis Wizard

Wizard displays sensorgrams, fitted curves and apparent kinetic constants...



...generates a trend analysis of baseline level...



...and generates results from control experiments

Kinetic Analysis Wizard

With knowledge of the assay principle and sample details, the Kinetic Analysis Wizard begins by recommending an experimental plan. This Biacore Wizard works throughout the kinetic investigation to:

- calculate and display apparent kinetic constants, sensorgrams and fitted curves
- generate trend analyses of baseline levels
- generate results from selected control experiments

Preliminary data is immediately available after every run.

Binding Analysis Wizard

The Binding Analysis Wizard is a tool for those working with experiments that require simple YES/NO answers for binding events. To provide an overview of the results, specified report points are presented as bar charts in which selected phases of each cycle can be displayed. Typical examples of experiments to study single pair or multi-molecular binding include:

- epitope mapping
- study of multi-molecular complexes
- use of enhancement or confirmatory reagents

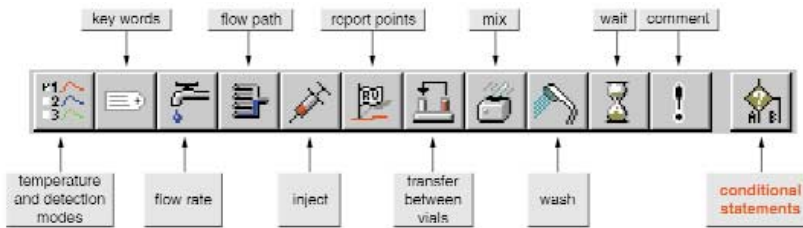
Develop specialized applications efficiently

Biacore 3000 offers the flexibility to develop methods for applications in which modification of standard procedures are required to achieve the optimum results.

Customized Application Wizard

This Biacore Wizard provides programming flexibility when more demanding methods are required. Customized experiments are programmed at the push of a button.

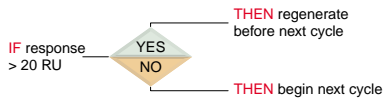
Fig. 12.



Conditional statements

Conditional Statements (IF/THEN) enable Biacore 3000 to respond correctly when specific predefined conditions are encountered. This can speed up analytical procedures by eliminating unnecessary steps, and the user can be sure that precious samples will be run only under satisfactory conditions. Examples of conditional statements are shown below:

Speed up procedures



Check surface conditions after regeneration



Fig. 11. Comparative results, displayed for each cycle, show YES/NO answers

Method Definition Language

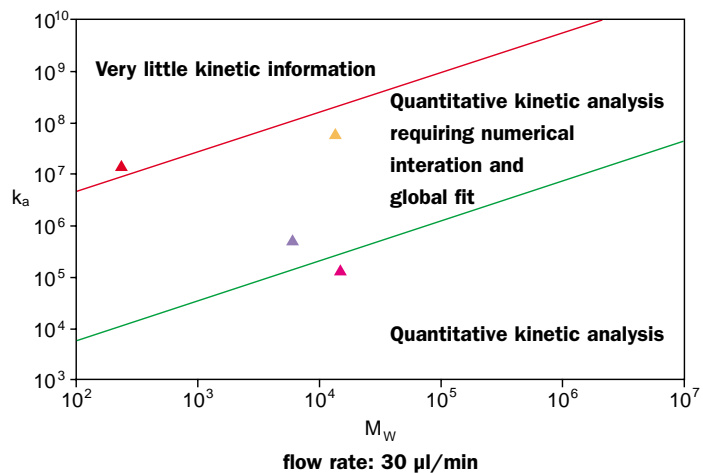
Highly specialized applications on Biacore 3000 may occasionally require fully customized methods. The Method Definition Language gives the user the complete freedom to develop a customized method step by step.

Perform advanced Kinetic evaluation

Biacore 3000 includes options for the most advanced kinetic evaluation, and performs affinity or concentration calculations. The superior performance of Biacore 3000 together with the global fit functions of BIAevaluation software further extends the range for quantitative kinetic analyses, allowing *confident interpretation of kinetic data* within a 10 RU range (shown in Figure 13).

Fig.13. Theoretical limits for kinetic analysis as a function of k_a and molecular weight under recommended experimental conditions. Actual experimental results also shown.

Kinetic information



Immobilized ligand	Analyte	MW Da (analyte)	k_a
1 Thrombin	Melagatran ▲	420	2×10^7
2 IgG	z-fragment ▲	8000	7.5×10^5
3 Anti-p24-Mab	p24 ▲	24000	2.2×10^5
4 Epo-receptor	Epo ▲	21000	8×10^7

Evaluation Wizards, tool tips and pre-programmed models reduce the need for mathematical expertise and accelerate the evaluation of even the most complex interactions. Biacore 3000 ensures that methods and results are easily controlled, evaluated and reported.

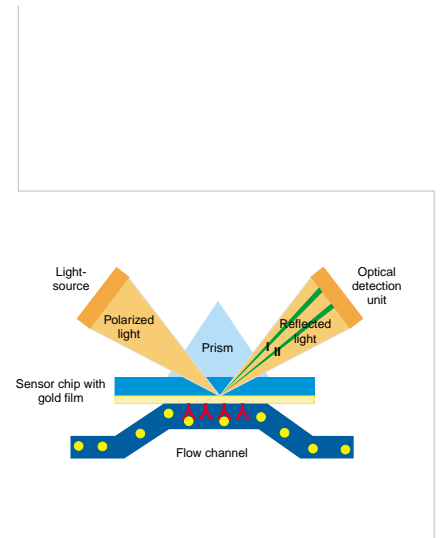
For the best results in biomolecular binding studies

- 1. expert control**
- 2. optimized monitoring**
- 3. advanced evaluation**

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*). 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 of protein 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 3000 is precisely controlled and primed to perfection to detect the smallest changes between a baseline signal and the signal generated when a second or third molecule interacts.



1. Control

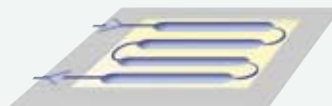
- Expert control system to run standard procedures optimally, with single analysis times of typically 1-5 minutes
- Binding events displayed in real time for direct assessment of kinetic constants and immediate feedback
- Programming flexibility for specialized applications
- Automation to shorten analysis times and increase throughput
- Sample recovery for complementary analysis

2. Monitor

- Temperature controlled detection environment together with an increased data acquisition rate result in a high signal to noise ratio and provide a relative working range of as little as 10 RU

2a. Microfluidic system

- Flow cells designed for studies from small molecules to whole cells, using < 5 µl of sample
- Four flow cells over one sensor surface to allow in-line reference subtraction from the same sample injection and ensure perfect controls
- Elimination of air/liquid interfaces to minimize protein denaturation
- Continuous injection to maintain sample concentration and ensure accurate rate constant determination
- Option to immobilize up to 4 different molecules
- Simultaneous multichannel detection to monitor up to 4 different interactions in a single injection
- Option to use paired flow cells for increased efficiency



2b. Sensor surfaces

- A wide selection of sensor surfaces to maximize application flexibility
- Highest quality sensor surfaces to minimize non-specific binding and give excellent reproducibility



3. Evaluate

- The most advanced tools for sophisticated, rapid data evaluation



System specifications

Sample handling:	automated sample loading and injection
Molecular weight detection:	>180 Da
Analytical performance	
<i>Myoglobin*</i> :	1 pM
Flow rate range:	1 - 100 µl/min, through flow cell, steps of 1 µl
Required sample volume:	injected volume + 20-80 µl, depending on application
Refractive index range:	1.33-1.40
Analysis temperature:	4 - 40°C (max 20°C below ambient)
Number of flow cells:	4 (used individually, in series or as 2 pairs)
In-line reference subtraction:	yes (flow cell 2-1, 3-1, 4-1, 4-3)
Dimensions (L x W x H):	760 x 350 x 610 mm
Electric voltage:	100-120 V; 220-240 V
Power consumption:	max 580 VA
Net Weight:	50 kg/110 lbs

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

Ordering information

Biacore 3000 system is delivered with processing unit, system controller, Biacore control, evaluation software, and Windows® 2000 operating system. Please contact your local Biacore representative for specified information.

Related products

BIAevaluation Software Kit BR-1002-16

Related information

Biacore Control Software Product Information Sheet BR-9001-28