Biacore® T100

Make critical decisions with confidence using protein interaction analysis

Define · Decide

BIACORE

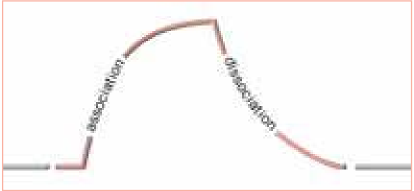
Base your decisions on the best

Information-rich protein interaction analysis

For more than 15 years Biacore systems have been providing scientists with exceptional insights into protein interactions.

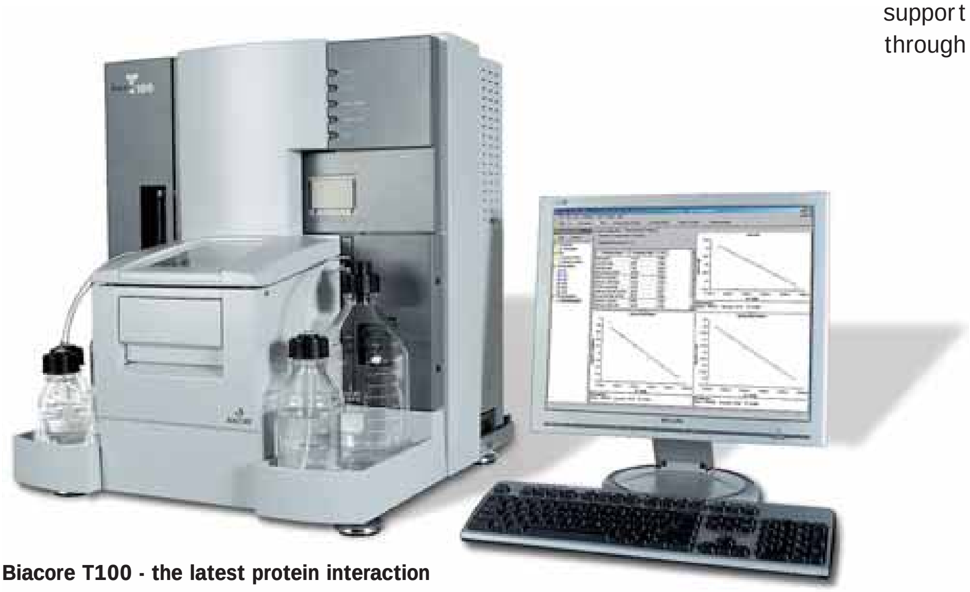
The characteristics of proteins are defined in terms of their specificity of interaction with other molecules, the rates at which they interact (association and dissociation) and their affinity (how tightly they bind to another molecule). The new Biacore T100 provides unmatched performance and adds yet another dimension, providing valuable information about the thermodynamic parameters involved in the interaction mechanism. Biacore's unique protein interaction analysis has a firm foundation in basic research and is used extensively in biotechnology and pharmaceutical companies. Biacore data enhances the quality of decisions at critical stages throughout the development and manufacture of novel therapeutics.

An excellent portfolio of Biacore systems ensures that you can choose the solution best suited to your needs.



A Biacore sensorgram:
No other techniques provide such comprehensive information in real time and from one system.

Make the most of protein interaction analysis. Obtain invaluable data to support your decisions from research through to QC.



Biacore T100 - the latest protein interaction analysis system from Biacore AB.

FACTS

Over 4000 peer-reviewed scientific publications have used Biacore systems to study protein interactions.

Biacore T100 - highest quality data in real time

Unmatched performance from research to QC

Biacore T100 at work
Now you can make critical decisions with confidence - from basic research, through drug discovery and development to manufacturing and QC. Biacore T100 enables you to determine specificity, affinity, kinetic and thermodynamic parameters, all in a single system. Base your decisions on the highest quality data.

- Elucidate disease mechanisms by characterizing native or recombinant protein interactions.
- Select the best antibodies as research tools, assay components or therapeutics by fully defining their interaction behavior.
- Define potential drug targets and diagnostic markers.
- Develop assays to characterize protein: LMW compound interactions in drug development.
- Select protein therapeutic candidates according to their on/off rates.
- Detect and characterize immune responses during preclinical and clinical development.
- Characterize protein therapeutics and implement QC methods.

BIACORE T100
21 CFR Part 11
COMPLIANCE

Welcome to the next generation of performance, flexibility and ease of use with Biacore T100.

Productive research and development
Biacore T100 incorporates the knowledge and experience gained since the launch of the very first Biacore system to provide you with a flexible, multi-application solution that sets the performance standard for protein interaction analysis. Software wizards assist with the analysis of every interaction parameter, making the system straightforward to use and suitable for both novice and experienced users. With Biacore T100, applications, such as antibody characterization, which could take weeks by conventional methods, can be completed in days.

Regulatory compliance
If your working procedures are subject to stringent regulatory requirements, an optional GxP support package will save you significant time during validation. Biacore T100 has been specifically designed with a high level of in-built GLP/GMP/GCP support for 21 CFR Part 11 compliance.

Choose the right antibody

Make well-informed selections based on high-resolution characterization

Antibodies and other proteins can be fully characterized within days using Biacore T100.

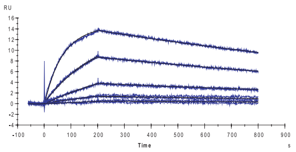
In basic and applied research, clearly defining interaction characteristics ensures that the best antibodies are selected as research tools or therapeutic candidates.

In the application described below, Biacore T100 enabled:

- Confident selection of therapeutic candidates.
- Better-informed assessment of the development process.
- Robust analysis at 37°C for more physiologically relevant predictive value.
- Excellent correlation between off-rates and cell assay (results not shown).

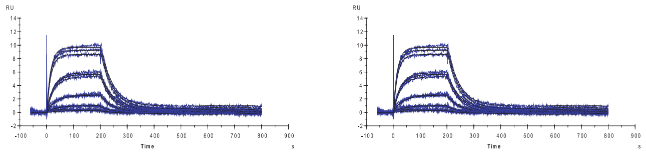
Selection of therapeutic candidates based on high-resolution kinetic analysis at physiological temperature

Original mouse Fab

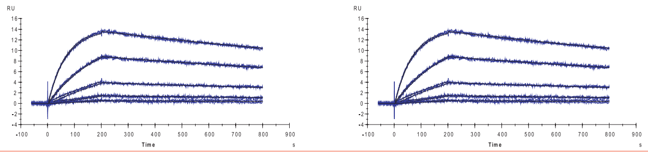


Analysis:
All assays run at 37°C. Evaluation wizards fit data and derive kinetic constants.

Candidates:
Engineered human Fabs derived from a mouse antibody against a protein involved in *P. aeruginosa* infections.

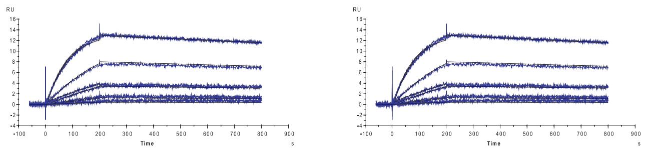


Human Fabs with a range of affinities compared to the starting mouse Fab.



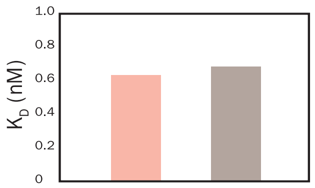
Conclusion:
Affinity differences mainly due to variations in off-rates (k_d values varied 100-fold compared to 10-fold for k_a values).

Matured Fabs:
Showed similar affinities to the best candidates from screening.



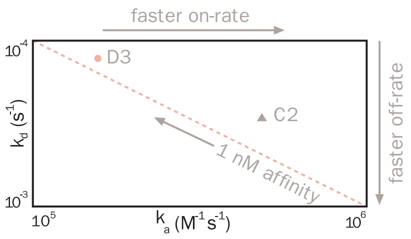
Conclusion:
Kinetics revealed that maturation produced Fabs with the slowest off-rates.

Affinity data would suggest equivalent therapeutic potentials and little or no effect of maturation process.



Fab C2
(from screening)

Fab D3
(matured from Fab C2)



Resolution of kinetic binding properties reveals significant differences in on- and off-rates.

Unique conclusion from Biacore T100:
Maturation produces slower off-rates better suited to this therapeutic application.

Data courtesy of D. Myszka, University of Utah and KaloBios Pharmaceuticals Inc. USA.

Comprehensive characterization of immune responses

Reliable, high information content assays for confident definition of serum antibody properties

Comprehensive characterization of immune responses is essential in immunotherapeutic and vaccine development, as well as in clinical immunogenicity studies.

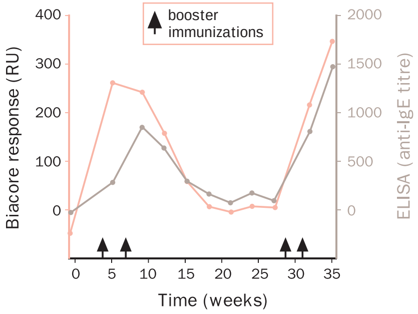
In this application, Biacore T100 offers a new approach for optimization of immunization regimes in immunotherapeutic development:

- Qualitative assessment of antibody binding stability over immunization period.
- Immune responses in relation to time, adjuvant and dosing regimes.
- IgG subclass determination (data not shown).
- Data consistent with ELISA but with much higher reproducibility and information content.

Characterizing immune responses in the development of immunization regimes for an immunotherapeutic protein

Immunotherapeutic: Recombinant IgE-derived protein designed to provoke an immune response against IgE, providing a novel therapy for allergy and asthma.

Experimental: Anti-IgE immune responses monitored over time in one primate using Biacore T100 and ELISA.



Response pattern: Peaks and declines after first two boosters and increases strongly again following 3rd and 4th boosters.

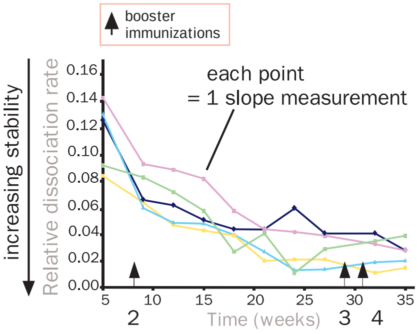
Comparison with ELISA: Anti-IgE response patterns correlated well for individual animals (earlier responses seen with Biacore in some cases).

Assay comparison: Anti-IgE ELISA results from Resistentia Pharmaceuticals AB.

	Biacore	ELISA
Intra-assay CV	0.35%	22%
Inter-assay CV	3.7%	38%

Excellent reproducibility compared to ELISA: Samples run in triplicate in five separate assays.

Maturation of immune response: Increasing stability was seen for all immune responses up to 20 weeks, but there was little further maturation after this time. The final two boosters had no effect on kinetic maturation of antibody responses.



Qualitative assessment of binding stability from real-time analysis: Relative slope calculations from early dissociation phases of binding curves enabled monitoring of relative antibody:antigen binding stability in relation to immunization regime.

Data courtesy of Resistentia Pharmaceuticals AB, Sweden.

Take interaction analysis beyond kinetics

Thermodynamics for better-informed prediction of structure-function relationships

Kinetic characterization gives information about the rate of complex recognition and stability, but to understand *why* the interaction proceeds at these rates, it is necessary to define the thermodynamics of the system.

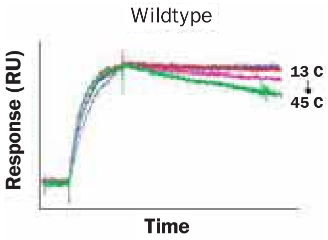
Fully understanding molecular recognition by being able to predict binding energetics through thermodynamic analysis can provide the basis for structure-based molecular design of drugs and engineered antibodies.

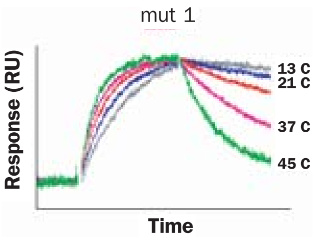
What do you need to know?	**Biacore T100 delivers**
Binding strength	**> Affinity constants**
Reaction rates	**> Kinetic rate constants**
Why reactants bind with a certain strength	**> Equilibrium thermodynamics**
Why complexes associate and dissociate at specific rates	**> Transition state thermodynamics**

Define the mechanisms that drive molecular interactions

Showing a case study on the interaction between hen egg lysozyme and single chain Fabs

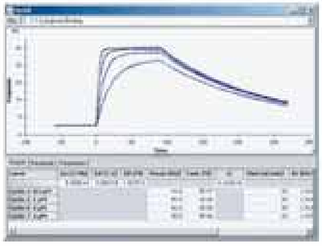
Kinetic analysis: Interaction between lysozyme and wildtype/mutant scFabs at different temperatures.

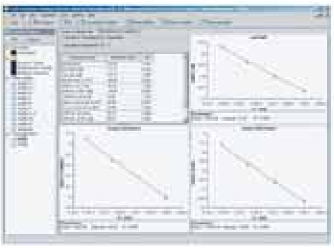




Highly stable experimental conditions made possible by the temperature controlled flow cell, sample storage compartment and sample degassing in Biacore T100.

Kinetic data is expressed in thermodynamic terms.

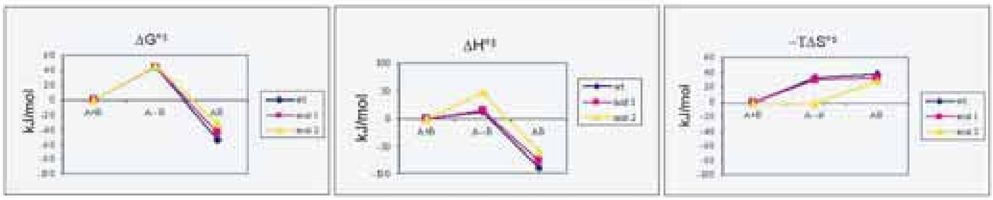




Evaluation wizards automatically fit data to thermodynamic equations.

Evaluate transition state thermodynamic parameters.

Key:
ΔG = free energy change
ΔH = enthalpy change
ΔS = entropy



Values for $\Delta G^{o\ddagger}$ may be obtained from experiments run at a single temperature but by obtaining kinetic rate constants at different temperatures, it is possible to resolve $\Delta G^{o\ddagger}$ into $\Delta H^{o\ddagger}$ and $\Delta S^{o\ddagger}$ terms.

Conclusion: Association of the mutated Fab, mut 2, with lysozyme proceeds according to a very different molecular mechanism from that governing either wild type or mut 1. The difference is apparent only if activation free energy, derivable using Biacore T100, is resolved into its enthalpic and entropic components, by performing kinetic analysis over a range of temperatures.

Reagents courtesy of Izumi Kumagai, Kouhei Tsumoto and Takeshi Nakashini, Tohoku University, Japan.

Biacore T100 - for protein interaction analysis

The key to critical decision-making from research to QC

Biacore would like to thank all our collaborators involved during the development of Biacore T100:
D. Myszka and J. Papalia, University of Utah; P. Flynn, K. Luehrsen and M. Baer of KaloBios Pharmaceuticals Inc.; S. Persson and M. Fant of Resistentia Pharmaceuticals AB and Professor Izumi Kumagai, Dr Kouhei Tsumoto and Dr Takeshi Nakashini, Tohoku University.

Among these collaborators, Biacore T100 has already earned a solid reputation for delivering unmatched performance and unique, real time data.

“We find Biacore T100 to be exceptionally easy to run, with intuitive operating and analysis software.

The ability to collect high quality data at elevated temperatures and to automatically switch between multiple buffer systems expands the application of Biacore as a biophysical tool for characterizing protein interactions.”

David Myszka, PhD,
Director, Center for Biomolecular Interaction Analysis,
University of Utah, USA.

“Biacore provides the best available analysis of affinity and binding kinetics for our protein therapeutic candidates.”

Peter Flynn, PhD, Director, Biochemistry Research,
KaloBios Pharmaceuticals Inc., USA.

“I strongly believe that this application of protein interaction analysis could play a very important role in preclinical and clinical research programs for novel immunotherapies and vaccines, enabling better treatment regimes for patients and reduced cost of goods for pharmaceutical companies.”

Stefan Persson, PhD, Vice-President,
Safety Pharmacology and Toxicology,
Resistentia Pharmaceuticals AB, Sweden.

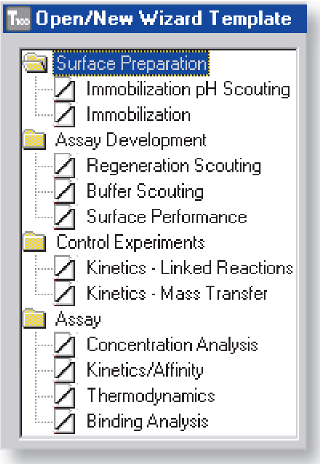
Highest quality data at your fingertips

Welcome to the new generation of performance, flexibility and ease of use

An intuitive user interface with control and evaluation wizards at every stage makes protein interaction analysis easier than ever.

Evaluation wizards for every interaction parameter give you results within seconds.

Develop, prepare and perform an analysis with control software wizards leading you step by step



- Surface Preparation
  - Immobilization pH Scouting
  - Immobilization
- Assay Development
  - Regeneration Scouting
  - Buffer Scouting
  - Surface Performance
- Control Experiments
  - Kinetics - Linked Reactions
  - Kinetics - Mass Transfer
- Assay
  - Concentration Analysis
  - Kinetics/Affinity
  - Thermodynamics
  - Binding Analysis

Guided immobilization of one interaction partner to a sensor chip surface.

Tools for easy optimization of assay conditions.

Guided control experiments for kinetic assays.

Step-by-step guidance for efficient set-up and running of protein interaction assays.

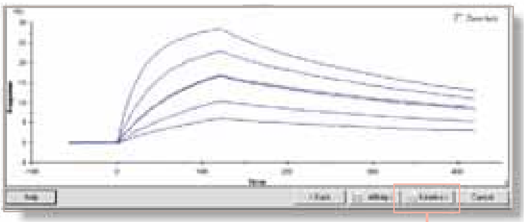
Each wizard offers a high level of guidance and support.

Interaction information:

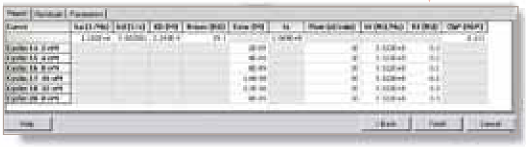
- Quantitative kinetics (rate constants)- over the broadest kinetic range
- Quantitative determination of affinity
- Concentration determination
- Determination of binding specificity (yes/no)
- Thermodynamic measurements
- Rapid epitope mapping

A few clicks and your evalution is complete

Open results file and select data required.



Choose evaluation wizard (data fitted to selected interaction model).



All kinetic parameters and relevant assay details displayed within seconds.

Ideal for work in regulated environments

Comprehensive compliance support saves time during validation

Analyze up to 384 samples automatically.

Use buffer scouting to speed up assay development or compare the effect of buffer components on interaction parameters.

Follow interactions at physiological temperatures to improve predictability of *in vivo* behavior.

Determine thermodynamic parameters of an interaction.

Benefit from additional support for assay development, analysis and evaluation when working with LMW compounds <1000 Da.

With excellent temperature control and integrated buffer degassing, Biacore T100 ensures reproducible results when working at elevated temperatures or with temperature-sensitive samples.

Biacore T100 GxP Package

For those working in GLP/GMP/GCP environments, the addition of a Biacore T100 GxP Package will ensure the highest level of GxP support and 21 CFR Part 11 compliance.

This package includes:

- Biacore T100 GxP Software (requires Windows® 2000 or Windows® XP Professional and includes Biacore T100 GxP Control Software and Biacore T100 GxP Evaluation Software)
- Biacore T100 GxP Handbook
- Instrument and Software Handbook
- Validation Support

Validation Support for Biacore T100

- 21 CFR Part 11 assessment report
- Software conformance certificate
- Hardware conformance certificate
- Supplementary documentation and services (upon request)
  - ESCROW agreement
  - On-site audit of Biacore AB
- GxP Services including Equipment Qualification

Equipment Qualification and Training

Equipment Qualification and training are performed by GMP-trained, qualified Biacore personnel when the system is installed in its selected operating environment. Our Equipment Qualification Services meet worldwide regulatory expectations and include:

- IQ/OQ (Installation Qualification/ Operational Qualification)
- IPQ (Initial Performance Qualification)
- Preventive Maintenance (PM) GxP

Sensor chip selection guide

Right system - right sensor surface

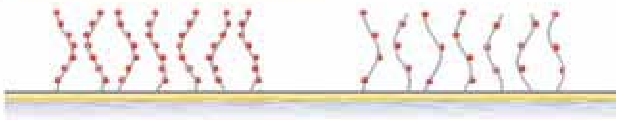
You've chosen the right system - now choose the right sensor surface

To study an interaction, one of the interaction partners is immobilized onto the sensor surface of a Biacore sensor chip. Immobilization occurs by direct coupling to the surface or via a capture molecule coupled to the surface.

A range of sensor chips ensures that the most suitable sensor surface can be chosen according to the nature of the molecule to be coupled and the requirements for the analysis.

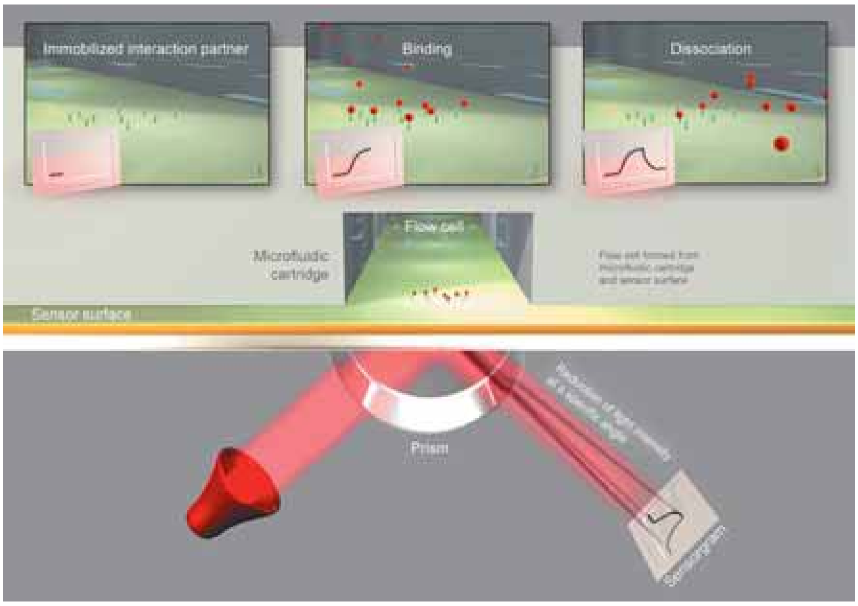
- and the right reagents and buffers

For a complete listing of reagents, buffers and accessories designed to ensure optimum performance with Biacore T100, refer to your Biacore catalog or visit www.biacore.com.

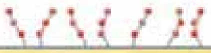
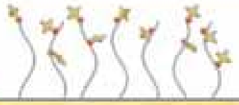


Molecule to be immobilized	Series S Sensor Chip CM5		Series S Sensor Chip CM4	
Proteins	●	The first choice for immobilization of proteins via $-NH_2$, -SH, -CHO, -OH, -COOH	◒	Useful if contaminants have a high positive charge
Tagged proteins	●	For GST-tags (surface is derivatized with anti-GST antibody)		
LMW molecules, typically <1000 Da	●		◒	
Membrane–associated molecules				
Nucleic acids	●	When modified with an amine group	◒	Useful if contaminants have a high positive charge
Carbohydrates	●	When derivatized with an aldehyde group	◒	Useful if contaminants have a high positive charge
Viruses or intact cells				

● Recommended choice ◒ Good alternative



Real-time protein interaction analysis utilizes the phenomenon of surface plasmon resonance.

Series S Sensor Chip CM3	Series S Sensor Chip C1	Series S Sensor Chip SA	Series S Sensor Chip HPA	Series S Sensor Chip L1	Series S Sensor Chip NTA
					
◒ If partner in solution is very large e.g. a molecular complex	◒ If partner in solution is multivalent or very large e.g. a molecular complex	● When biotinylated			
					● For His-tags
◒					
			● Incorporate molecule into a lipid monolayer	● Incorporate molecule into a lipid bilayer	
◒ If partner in solution is very large e.g. a molecular complex	◒ If partner in solution is multivalent or very large e.g. a molecular complex	● When biotinylated			
◒ If partner in solution is very large e.g. a molecular complex	◒ If partner in solution is multivalent or very large e.g. a molecular complex	● When biotinylated			
● Keep large particles close to the surface to maintain sensitivity	● Keep large particles close to the surface to maintain sensitivity				

Recommendations in this table are based on the experience of scientists who have studied thousands of different protein interactions since the first Biacore system was introduced.

Protein interactions are monitored by injecting samples over the prepared surface of a sensor chip. Between injections, the surface is regenerated by selective dissociation of the interaction partners.

Visit www.biacore.com to learn more about protein interaction analysis.

Series S Sensor Chips for Biacore T100

Certified sensor chips are the first choice for use in regulated environments. All certified Series S Sensor Chips are subjected to the highest stringency of QC and delivered with a Certificate of QC. A non-certified Series S Sensor Chip CM5 is also available.

BR-9004-15

Service and support

Getting the most out of your Biacore system

Biacore offers a comprehensive range of service programs, support tools and information services. Our goal is to provide you with the optimum level of support so that your Biacore system continues to make a key contribution to your work.

Online Support including:

- BIAsimulation to perform dry-run experiments to verify data or as a learning tool for protein interaction analysis.
- Tech Tips & Protocols designed to enhance the effectiveness of your Biacore system.
- Immobilization and regeneration database to quickly find an immobilization or regeneration protocol relevant to the interaction partners you are investigating.

Instrument Service

- Service information and specific system service contracts.

Validation Support

- Validation and qualification services to meet regulatory requirements.

Training

- Including Getting Started Kits and interactive CDs.
- Training courses for both new and more experienced users.

Publications

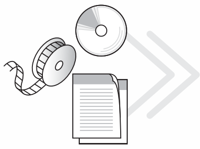
- Search reference database containing thousands of peer-reviewed papers citing Biacore.

Software Downloads

- Download the latest version of your software.
- Download and review software notes, technical details and the latest news about your software.

MSDS

- Find Material Safety Data Sheets relating to Biacore consumables.



Visit www.biacore.com or talk to your local Biacore representative for more information.

Define · Decide

BIACORE

PRODUCT INFORMATION

Biacore T100

Unmatched performance for protein interaction analysis: From basic research to QC

- **Highest quality data in real time**
  - Analyze up to 384 samples per run, automatically
  - Optimized for highest quality kinetics, from the fastest on-rates to the slowest off-rates
  - Work with low molecular weight compounds
  - Study interactions at physiological temperatures and above
  - Sample recovery for identification by mass spectrometric analysis
- **Use software wizards for fast assay development, analysis and evaluation of every interaction parameter**
  - Evaluate kinetics, affinity, concentration, specificity and thermodynamics with a few simple clicks
- **Work in GxP-regulated environments using Biacore T100 GxP Package**
  - Validated software supporting GxP-regulated working processes and including technical controls for 21 CFR Part 11 compliance
  - Validation support including Equipment Qualification (IQ/OQ/IPQ)

Biacore® T100 is a system for comprehensive protein interaction analysis from early research, to drug discovery and development and on to QC. The system is supported by state of the art software for assay development, analysis, data evaluation and interpretation. Software wizards ensure ease of use. Alternatively, methods may be developed or customized as required. In addition to providing detailed information on kinetics and affinity, software support allows interactions to be thermodynamically characterized.

With an optional GxP package, Biacore T100 can be operated in compliance with current GxP regulations.

Biacore T100 includes a processing unit, a computer with pre-installed control and evaluation software and Windows® operating system. The system is delivered with Instrument and Software Handbooks.

Define · Decide

BIACORE

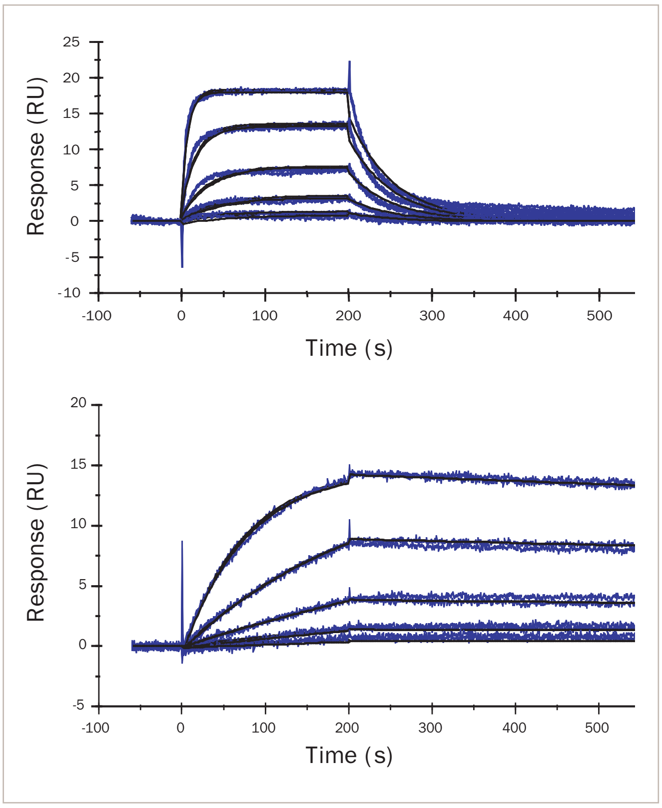
Highest quality data in real time

Analyze up to 384 samples per run

- Supports the use of 96 and 384 well microplates
- For a typical analysis of 7 minutes per cycle, up to 384 samples may be processed during 48 hours of unattended operation in a single run
- Sample cooling in a temperature-controlled sample compartment facilitates unattended analysis of temperature-sensitive samples

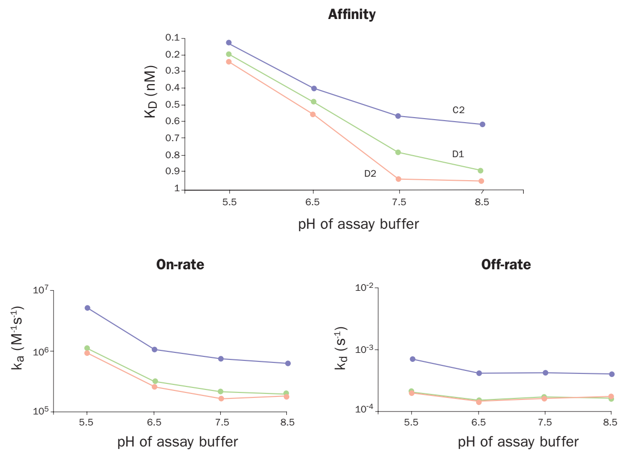
Optimized for highest quality kinetics

- Four flow cells for single, paired or serial runs
- Paired, on-chip flow cell connections give minimum void volume between flow cells, ensuring the best possible conditions for accurate reference subtraction
- Measure kinetic rate constants over the broadest range, from the fastest on-rates to the slowest off-rates
    - On-rates from 10^3 to 10^7 $M^{-1}s^{-1}$ (and higher for macromolecular analytes)
    - Off-rates from 10^{-5} to 0.5 s^{-1}



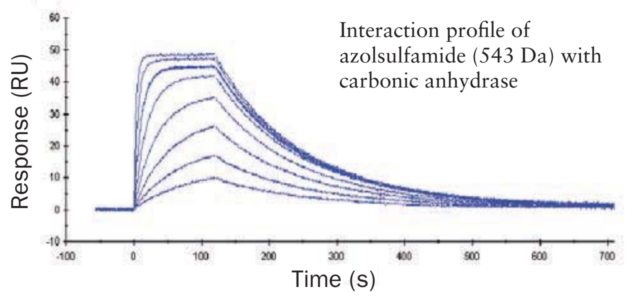
Compare buffer effects in a single run

- Use buffer scouting for fast assay development; up to 4 different buffers can be tested at one time
- Study micro-environmental effects on binding properties in mechanistic and stability studies
- Define the kinetic properties of protein samples in varied biochemical/biophysical environments. This information may be crucial when selecting candidates intended for use in the complex and variable environment of clinical treatment



Buffer scouting shows that the affinities of interactions between an antigen and 3 different Fab fragments increase in buffers below pH 7.5

Study interactions involving low molecular weight compounds



Interaction profile of azolsulfamide (543 Da) with carbonic anhydrase

- Investigate interactions involving binding partners with molecular weight as low as 100 Da
- Dedicated software supports kinetic evaluation of LMW compound interactions

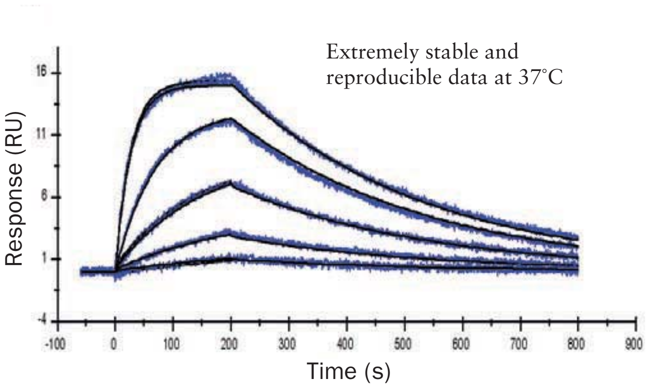
Study interactions at physiological temperatures and above

Temperature controlled flow cell and sample compartment:

- Allows the analysis of temperature sensitive samples and the study of interactions at temperatures from 4°C to 45°C
- Allows interactions to be studied at physiological temperatures, enabling the behavior of therapeutics in vivo to be more confidently predicted

Integrated buffer degasser:

- Eliminates the appearance of air bubbles in the flow system, allowing analysis of samples at higher temperatures
- Improves robustness when studying interactions at elevated temperatures



Extremely stable and reproducible data at 37°C

Sample recovery for identification by mass spectrometric analysis

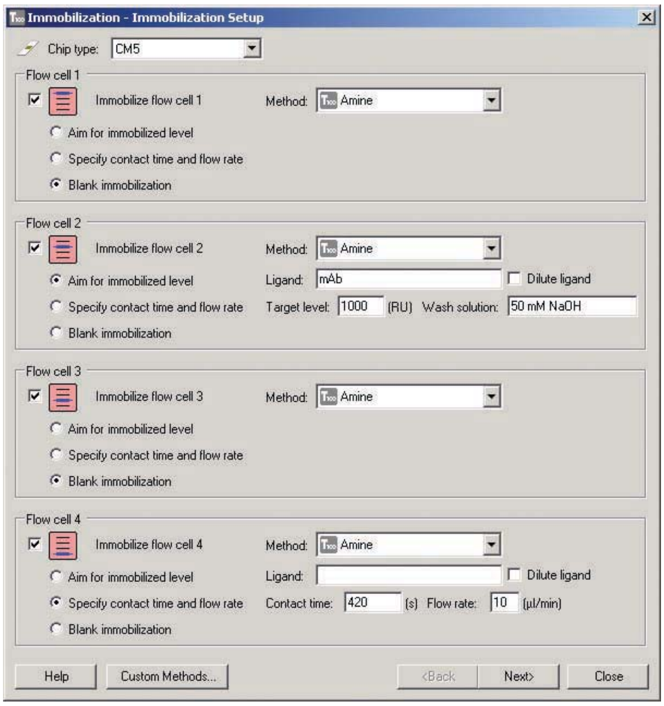
Protein interaction analysis on Biacore T100 in combination with mass spectrometry provides the possibility to identify proteins on the basis of functional binding criteria. Sample recovery and subsequent digestion are supported by the system software.

- Analytes recovered in a small volume, maximizing the concentration
- Minimum carry-over from sample to recovered solution
- Deposition in vial containing digestion solution
- Entire recovery process defined in Method builder software in predefined template

Software wizards for fast assay development, analysis and evaluation

Immobilization support for various chemistries and sensor surfaces

- Use pre-defined methods to immobilize interaction partners via amine, thiol, aldehyde or maleimide chemistries
- Support for customized immobilization methods



Immobilization - Immobilization Setup

Chip type: CM5

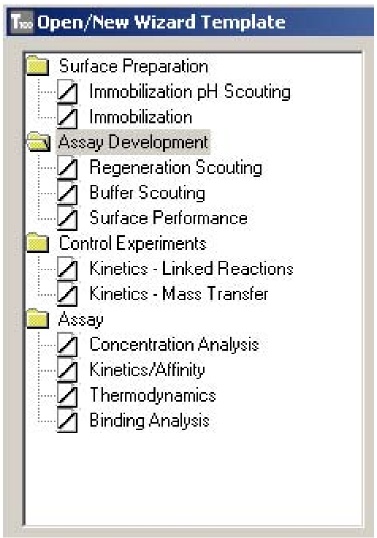
Flow cell 1
☑ Immobilize flow cell 1 Method: Amine
○ Aim for immobilized level
○ Specify contact time and flow rate
● Blank immobilization

Flow cell 2
☑ Immobilize flow cell 2 Method: Amine
● Aim for immobilized level Ligand: mAb ☐ Dilute ligand
○ Specify contact time and flow rate Target level: 1000 (RU) Wash solution: 50 mM NaOH
○ Blank immobilization

Flow cell 3
☑ Immobilize flow cell 3 Method: Amine
○ Aim for immobilized level
○ Specify contact time and flow rate
● Blank immobilization

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

- Software wizards support all steps in assay development

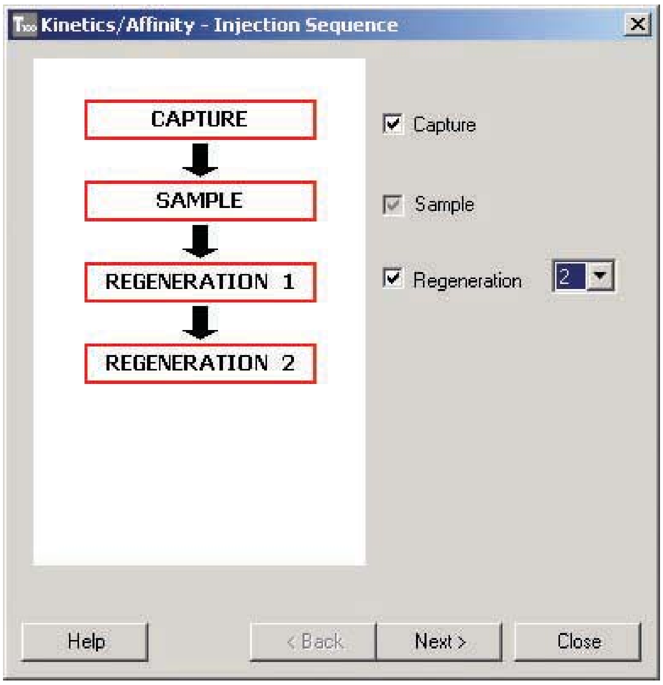


Open/New Wizard Template

- Surface Preparation
  - Immobilization pH Scouting
  - Immobilization
- Assay Development
  - Regeneration Scouting
  - Buffer Scouting
  - Surface Performance
- Control Experiments
  - Kinetics - Linked Reactions
  - Kinetics - Mass Transfer
- Assay
  - Concentration Analysis
  - Kinetics/Affinity
  - Thermodynamics
  - Binding Analysis

Characterize interactions in terms of kinetics and affinity

- Set up assays quickly and easily using wizard support

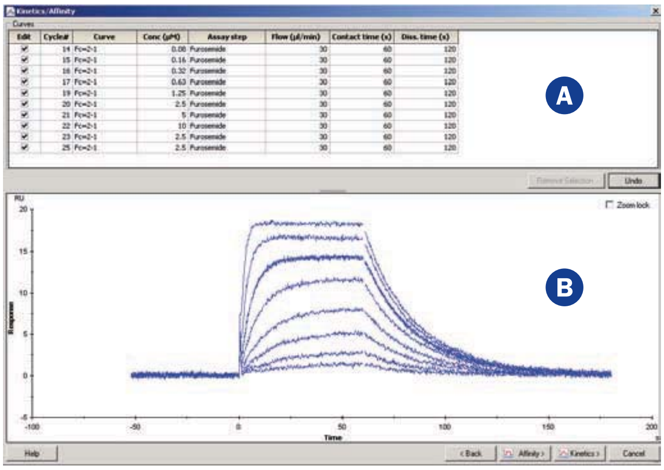


Kinetics/Affinity - Injection Sequence

CAPTURE → SAMPLE → REGENERATION 1 → REGENERATION 2

☑ Capture
☑ Sample
☑ Regeneration 2

- Perform kinetic evaluation of interactions with a few simple clicks
- Fit the kinetic data using one of several available interaction models
- Automatically subtract reference and blank data



Kinetics/Affinity

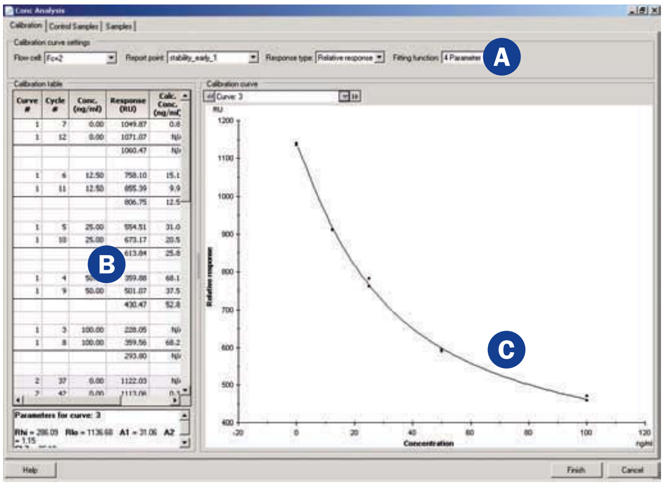
Curves

Edit	Cycle#	Curve	Conc (µM)	Assay step	Flow (µl/min)	Contact time (s)	Diss. time (s)
✓	14	Fc=2-1	0.08	Furosemide	30	60	120
✓	15	Fc=2-1	0.16	Furosemide	30	60	120
✓	16	Fc=2-1	0.32	Furosemide	30	60	120
✓	17	Fc=2-1	0.63	Furosemide	30	60	120
✓	19	Fc=2-1	1.25	Furosemide	30	60	120
✓	20	Fc=2-1	2.5	Furosemide	30	60	120
✓	21	Fc=2-1	5	Furosemide	30	60	120
✓	22	Fc=2-1	10	Furosemide	30	60	120
✓	23	Fc=2-1	2.5	Furosemide	30	60	120
✓	25	Fc=2-1	2.5	Furosemide	30	60	120

A Data fitted to selected interaction model
B All kinetics parameters displayed within seconds

Concentration

- Measure active concentration, not just total protein, using software-supported direct binding and inhibition assays
- Monitor sensor surface stability via repeated calibration curves, ensuring reliable data from run to run
- Ensure rigorous quality control by inclusion of control samples
- Have confidence in data by comparing measured and predicted concentrations generated by the evaluation software



Flow cell: Fc=2 Report point: stability_early_1 Response type: Relative response Fitting function: 4 Parameter

Curve #	Cycle #	Conc. (ng/ml)	Response (RU)	Calc. Conc. (ng/mL
1	7	0.00	1049.87	0.8
1	12	0.00	1071.07	N/
			1060.47	N/
1	6	12.50	758.10	15.1
1	11	12.50	855.39	9.9
			806.75	12.5
1	5	25.00	554.51	31.0
1	10	25.00	673.17	20.5
			613.84	25.8
1	4	50	359.88	68.1
1	9	50.00	501.07	37.5
			430.47	52.8
1	3	100.00	228.05	N/
1	8	100.00	359.56	68.2
			293.80	N/
2	37	0.00	1122.03	N/

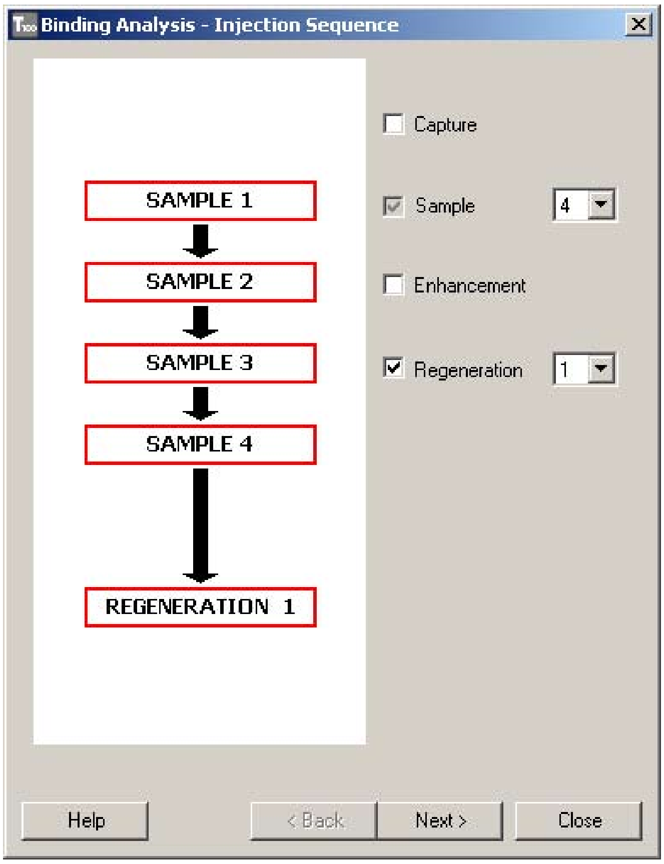
Parameters for curve: 3

Rhi = 206.09 Rlo = 1136.68 A1 = 31.06 A2 = 1.15

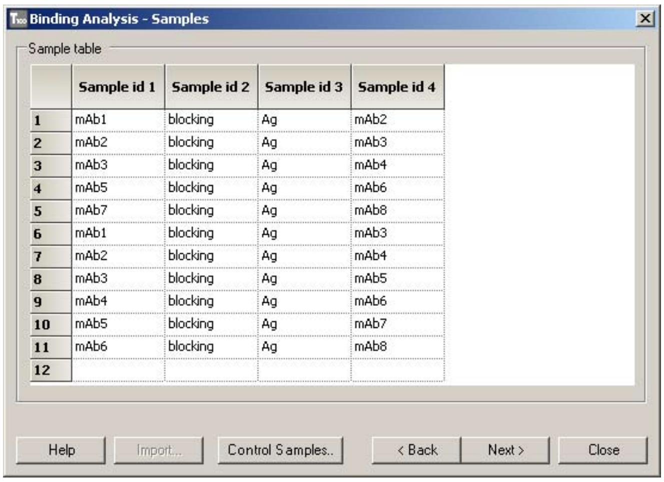
A Linear or four-parameter fitting
B Concentration and %CV displayed
C Calibration curve

Specificity

- Study up to four interactions simultaneously (three interaction partners and a reference surface)
- Rank binders or obtain simple yes/no answers in applications such as epitope mapping



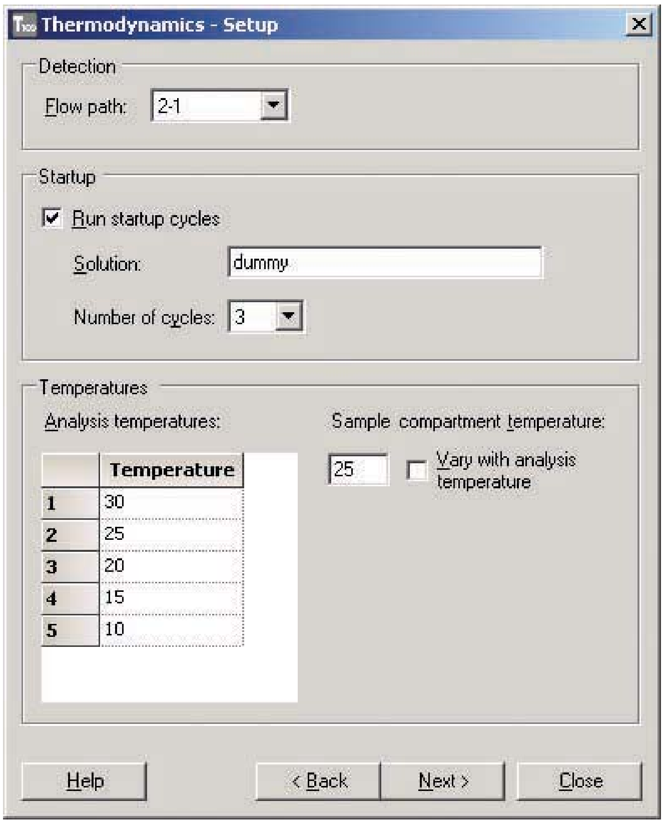
☐ Capture
☑ Sample 4
☐ Enhancement
☑ Regeneration 1



	Sample id 1	Sample id 2	Sample id 3	Sample id 4
1	mAb1	blocking	Ag	mAb2
2	mAb2	blocking	Ag	mAb3
3	mAb3	blocking	Ag	mAb4
4	mAb5	blocking	Ag	mAb6
5	mAb7	blocking	Ag	mAb8
6	mAb1	blocking	Ag	mAb3
7	mAb2	blocking	Ag	mAb4
8	mAb3	blocking	Ag	mAb5
9	mAb4	blocking	Ag	mAb6
10	mAb5	blocking	Ag	mAb7
11	mAb6	blocking	Ag	mAb8
12				

Derive transition state thermodynamics from kinetic rate constants

- Dedicated evaluation software wizards, in-built buffer degassing and temperature control of Biacore T100 make transition state thermodynamic analysis easier than ever
- Integration of rate constants measured across several temperatures into thermodynamic equations allows thermodynamic characterization of transition state, revealing the forces driving the interaction
- Equilibrium thermodynamic parameters, ΔH and ΔS, are in close agreement with those obtained using isothermal titration calorimetry



Flow path: 2-1

☑ Run startup cycles

Solution: dummy

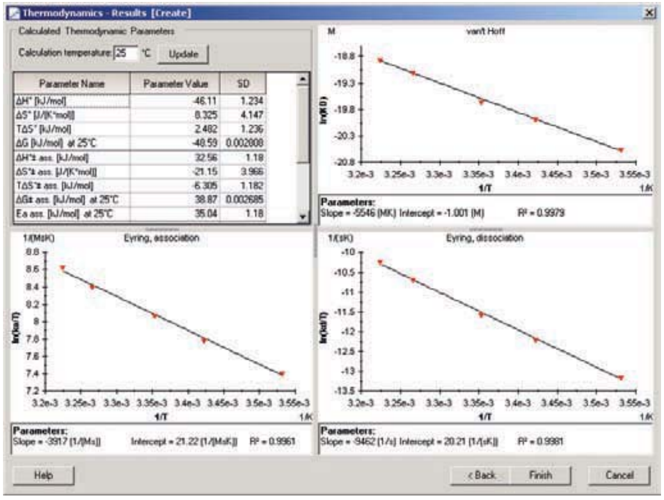
Number of cycles: 3

Analysis temperatures:

	Temperature
1	30
2	25
3	20
4	15
5	10

Sample compartment temperature: 25 ☐ Vary with analysis temperature

1. Run kinetics at varying temperatures



Calculation temperature: 25 °C

Parameter Name	Parameter Value	SD
ΔH° [kJ/mol]	-46.11	1.234
ΔS° [J/(K*mol)]	8.325	4.147
$T\Delta S^\circ$ [kJ/mol]	2.482	1.236
ΔG [kJ/mol] at 25°C	-48.59	0.002808
$\Delta H^{\circ\ddagger}$ ass. [kJ/mol]	32.56	1.18
$\Delta S^{\circ\ddagger}$ ass. [J/(K*mol)]	-21.15	3.966
$T\Delta S^{\circ\ddagger}$ ass. [kJ/mol]	-6.305	1.182
$\Delta G^\ddagger$ ass. [kJ/mol] at 25°C	38.87	0.002685
Ea ass. [kJ/mol] at 25°C	35.04	1.18

Parameters:
Slope = -5546 (MK) Intercept = -1.001 (M) R² = 0.9979

Parameters:
Slope = -3917 (1/(Ms)) Intercept = 21.22 (1/(MsK)) R² = 0.9961

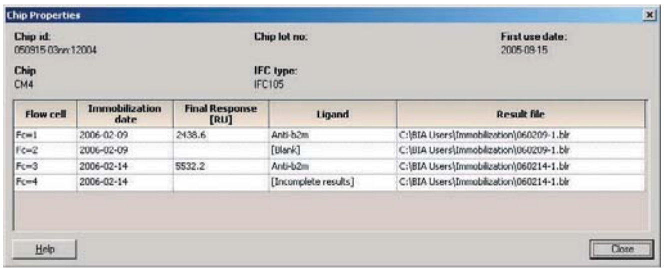
Parameters:
Slope = -9462 (1/s) Intercept = 20.21 (1/(sK)) R² = 0.9981

2. Automatic generation of Eyring and van't Hoff plots from kinetic data for calculation of thermodynamic parameters

Keep track of chip information using Chip Log

Chip Log records information on immobilization, which is exported together with result files to hard drives, databases or servers. Chip Log:

- Displays the properties of the sensor chip
- Simplifies multiple, interrupted usage of a single sensor chip
- Records information on chip usage – an important issue if multiple users have access to the instrument



Chip id:
050915 03nn:12004

Chip lot no:

First use date:
2005-09-15

Chip
CM4

IFC type:
IFC105

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

Work in GxP-regulated environments

Biacore T100 GxP Package *(optional)*

For those working in GLP/GMP/GCP environments, the addition of a Biacore T100 GxP Package will ensure the highest level of GxP support and 21CFR Part 11 compliance. The validated software meets 21 CFR Part 11 compliance for electronic records.

Features include:

- Data integrity – access control and enforced version handling
- User authorization levels – administrator, developer and user levels set access rights to software functions
- Published procedures for operational control – enables run and evaluation settings to be locked together in routine assays
- Audit trails – tracks record modifications and maintains complete version histories for published procedures
- Data can be exported both manually and automatically in Microsoft Excel (xls) format as well as xml format

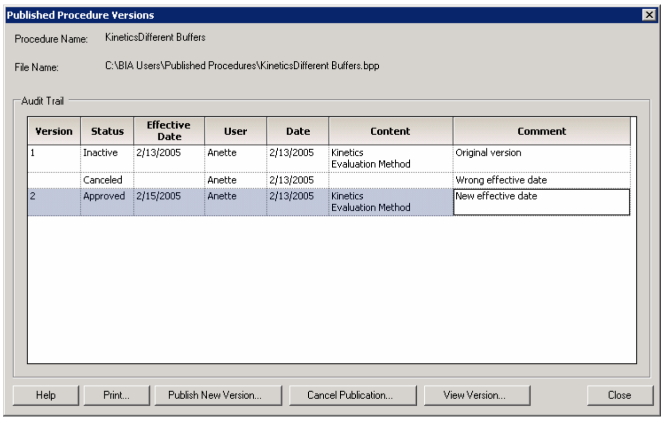
Validation support for full GxP compliance

In addition to the GxP software, validation support for Biacore T100 includes

- GxP documentation
  - System assessment report
  - Software conformance certificate
  - Hardware conformance certificate
  - Recommendations of OS configuration for 21 CFR Part 11 compliance
- GxP services
  - Instrument qualification (IQ/OQ/IPQ)
  - Preventative maintenance (PM) GxP
  - Requalifications
- Supplementary documentation and services available on request e.g.
  - ESCROW agreement
  - On-site audit of Biacore AB

Equipment qualification

Equipment qualification is performed by GxP-trained, qualified Biacore personnel when the system is installed in its selected operating environment. Our equipment qualification services meet worldwide regulatory expectations and include IQ/OQ (installation qualification/operational qualification), IPQ (initial performance qualification) and preventive maintenance (PM) GxP.



Published Procedure Versions

Procedure Name: KineticsDifferent Buffers

File Name: C:\BIA Users\Published Procedures\KineticsDifferent Buffers.bpp

Audit Trail

Version	Status	Effective Date	User	Date	Content	Comment
1	Inactive	2/13/2005	Anette	2/13/2005	Kinetics Evaluation Method	Original version
	Canceled		Anette	2/13/2005		Wrong effective date
2	Approved	2/15/2005	Anette	2/13/2005	Kinetics Evaluation Method	New effective date

Audit trail tracks record modifications and maintains complete version histories for published procedures.

Technical information and specifications

Detection technology	Surface Plasmon Resonance (SPR) biosensor
Information provided	Kinetic and affinity data (K_D, k_a, k_d), specificity, concentration and thermodynamics data
Data presentation	Result tables, result plots and real time monitoring of sensorgrams
Analysis time per cycle	Typically 2–15 min
Automation	48 hours unattended operation
Sample type	Low MW drug candidates to high molecular weight proteins (also DNA, RNA, polysaccharides, lipids, cells and viruses), in various sample environments, e.g. in DMSO-containing buffers, plasma, serum
Required sample volume	Injection volume + 20–50 µl (application dependent)
Injection volume	2–350 µl
Flow rate range	From 1–100 µl/min
Flow cell volume	0.06 µl
Flow cell height	40 µm
Sample/reagent capacity	1 × 96, or 384 well microplate + up to 33 reagent vials
Analysis temperature range	4–45 °C (maximum 20 degrees below ambient temperature)
Sample storage	4–45 °C (maximum 15 degrees below ambient temperature)
Sample refractive index range	1.33–1.39
Buffer selector	Automatic switching between 4 buffers
In-line reference subtraction	Automatic
Typical working ranges	
Kinetics:	
Association rate constant (k_a)	10^3 – 10^7 $M^{-1}s^{-1}$ (and higher for macromolecular analytes)
Dissociation rate constant (k_d)	10^{-5} – 0.5 s^{-1}
Sample concentration	10^{-3} – 10^{-11} M
Molecular weight detection	>100 Da
Number of flow cells	4
Baseline noise	Typically <0.1 RU (RMS)
Baseline drift	Typically <0.3 RU/min
Recovery specifications	1.5 µl analyte recovery volume
Dimensions	600 × 615 × 690 mm
Net weight	Total: 60 kg
Mains requirements	Processing Unit and System Controller: Autorange 100-240 V ac (±10 %), 50-60 Hz, Class 1 equipment (protective earthing)
Power consumption	Processing Unit: max 6.3 A (at 100 V ac) System Controller: max 7.2 A (at 100 V ac)
Data handling and storage	
PC operating systems	Microsoft® Windows® 2000 or Windows® XP (Professional)
Interfacing	Possibilities for import of sample data and export of results, e.g. to and from LIMS
Compliance	
Safety standard	EU:EN61010-1(2001), EN61010-1-081 (2002) North America: UL61010A-1 CAN/ CSA-C22.2 No.1010.1
EMC	EN 61326-1 (1997), Amendment A1 (1998) and Amendment A2 (2001)
GxP	Technical controls for 21 CFR Part 11(ER) compliance Validation support incl. Equipment Qualification

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Define · Decide

BIACORE