Label-free interaction analysis

Biacore™ T200

Biacore T200 (Fig 1) is a versatile, label-free system for detailed studies of biomolecular interactions, from early research to drug discovery and development, and on to quality control (QC).

The system delivers high quality kinetic, affinity, concentration, specificity, selectivity, comparability, and thermodynamic interaction data – in real time with exceptional sensitivity. Interactions characterized by on- and off-rates at the extremities of the kinetic scale can be analyzed with great precision and confidence.

- Sensitivity that allows you to push the limits of label-free interaction analysis
- Analyze interactants ranging from ions to viruses
- Get to final results faster using guided workflows with built-in data quality assessments
- Run 384 samples unattended and quickly co-evaluate up to 5000 samples in a single evaluation

Biacore T200 provides a comprehensive suite of tools for setting up, executing, and evaluating biomolecular interaction experiments. Easy-to-use wizards guide you from experiment setup to data interpretation of common assays; alternatively, use customizable methods and fully flexible data evaluation tools for more challenging investigations. The system efficiently delivers high-quality information, whether it is for the characterization of single interaction partners, focused screening of hundreds of samples, or comparability assessment of biotherapeutics.

An optional GxP package supports Biacore T200 operation in compliance with regulatory demands.

Fig 1. Biacore T200 for high sensitivity, label-free interaction analysis.

Main applications

The performance and versatility of Biacore T200 provides several advantages for a large range of applications involving biomolecular interaction studies.

- Increase understanding of molecular mechanisms and structure-function relationships
- Select, characterize, and assess comparability of biotherapeutics
- Select and optimize lead compounds during drug discovery
- Detect and characterize antidrug antibodies (ADA) in immunogenicity studies
- Perform time- and cost-efficient concentration analysis

Pushing detection limits of label-free interaction analysis

The sensitivity of Biacore T200 enables the precise affinity analysis of the smallest organic compounds (Fig 2) and extends the range of kinetic values that can be precisely determined. Previously borderline data may thus be confidently interpreted, enabling kinetic analysis of the simplest analytes, as well as detection of low abundance proteins.

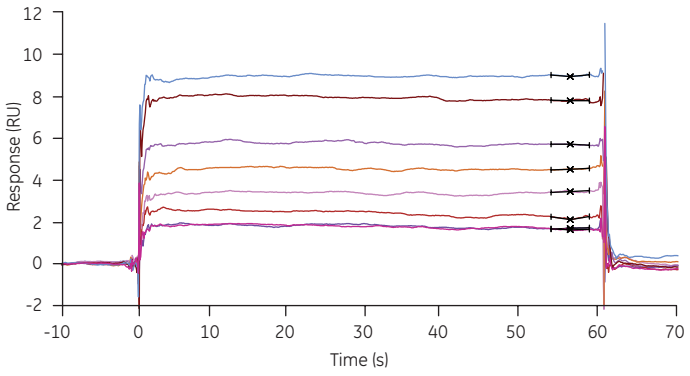

Fig 2. Interaction profile of methanesulfonamide (M_r 95) with carbonic anhydrase.

Biacore T200 is designed for the analysis of molecular interactions where high sensitivity is crucial:

- Small organic compounds (no minimum molecular weight limit)
- Low abundance molecules (concentration > 10 pM)
- Rare or sensitive targets such as G protein-coupled receptors
- Avoidance of avidity effects when analyzing interactions with bivalent antibodies
- Weak interactions, K_D in mM range
- Stable binders, $k_d \geq 10^{-5}$ s^{-1}

Working with rare or sensitive targets

The possibility to derive high quality data from low levels of immobilized proteins is advantageous in the analysis of interactions between small molecules and sensitive proteins such as G protein-coupled receptors (GPCRs), which are among the most important drug targets. The high sensitivity of Biacore T200 means that preservation of function is necessary in only a fraction of the total immobilized GPCRs (Fig 3). In addition, rare targets may be used sparingly, allowing for reduced consumption with no risk of compromise in data quality. Sensitive targets may thus be studied with greater confidence, reducing time-to-results in the drug discovery process.

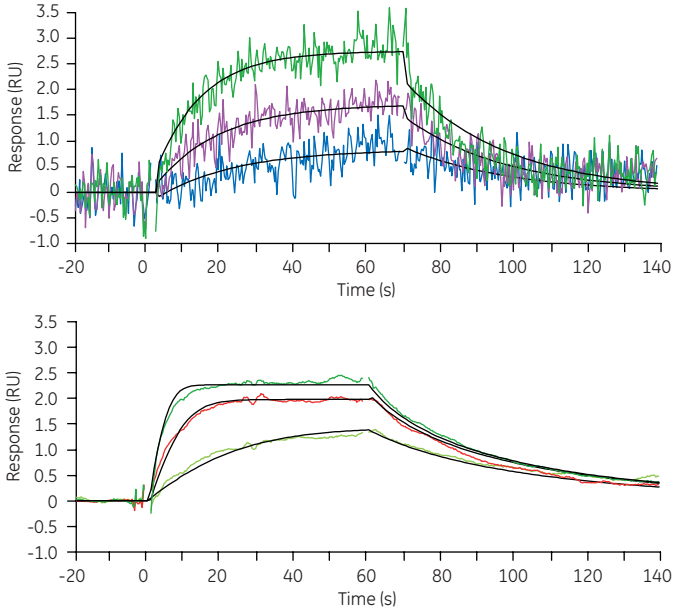

Fig 3. Binding of a small molecule, xanthine amine congener (XAC), to stabilized histidine-tagged GPCR (StaR™) A2. Data of higher quality is generated by Biacore T200 (lower sensorgram) compared to Biacore T100 (upper sensorgram) when using low levels of immobilized GPCR. Data courtesy of Dr. Andrei Zhukov, Heptares Therapeutics Ltd, Welwyn Garden City, UK.

Increased flexibility in assay design

With Biacore assays, it is usually preferable to immobilize antibody on the sensor surface, rather than in solution, in order to avoid complicating effects of avidity arising when antibodies attach to a surface densely coated with antigen.

The sensitivity of Biacore T200 is sufficient to allow antigen immobilization levels so low that avidity is avoided (Fig 4), thus adding full flexibility to assay design.

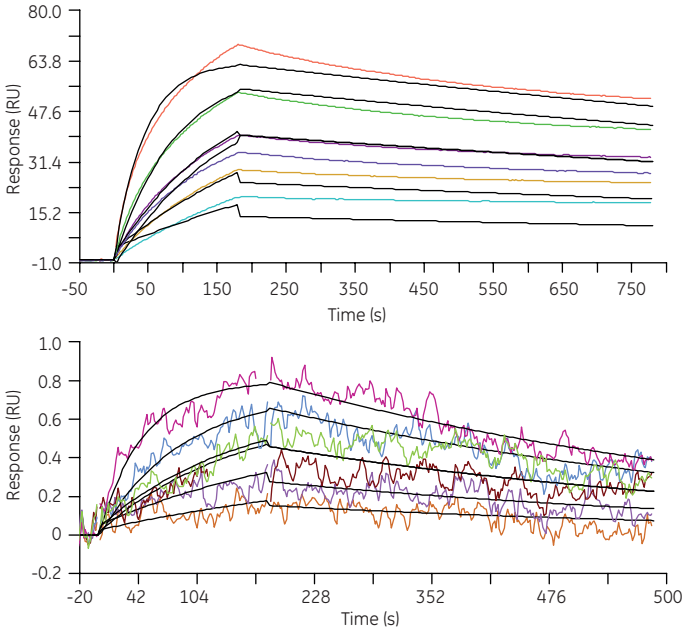

Fig 4. At low immobilization levels of antigen (lower sensorgrams), interaction data are better fitted to a 1:1 model. Kinetic rate constants of interactions involving antibodies as the binding partner in solution are thus calculated with greater precision and confidence.

High precision over a broad kinetic range

The microfluidic system in Biacore T200 is optimized for the highest quality kinetics. The four flow cells can be used for single, paired, or serial runs. Paired, on-chip flow cell connections lead to minimum void volume between flow cells, ensuring accurate reference subtraction.

Biacore T200 enables measurement of kinetic constants over a broad range, from really fast on-rates to very slow off-rates (Fig 5). This enables confident ranking of strong binders, which can be important in antibody selection. It also enables the detection of differences among rapid binders, an important feature when studying biological processes limited by bioavailability.

- k_a from 10^3 to 5×10^7 $M^{-1}s^{-1}$ (10^3 to 3×10^9 $M^{-1}s^{-1}$ for macromolecular analytes)
- k_d from 10^{-5} to 1 s^{-1}

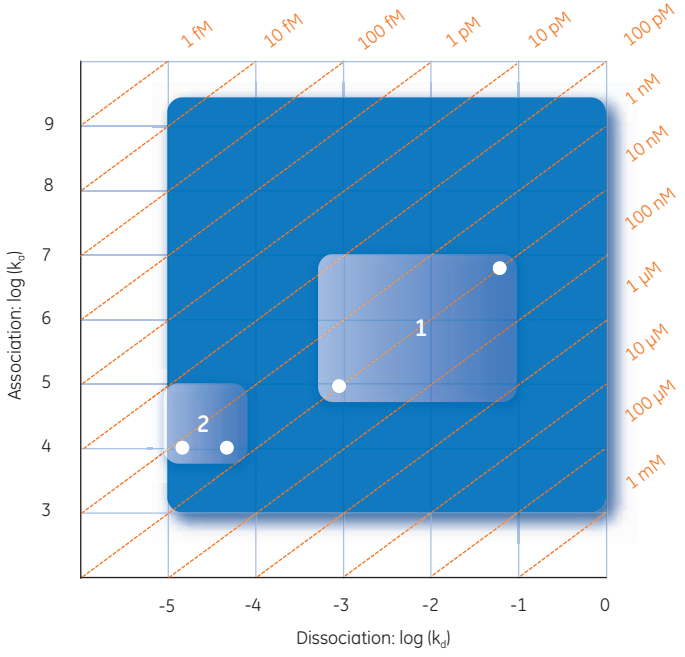

Fig 5. Kinetic measurements over a broad range, from really fast on-rates to very slow off-rates. (1) Interactions with apparently similar affinities can have very different kinetic profiles. Resolution into component on- and off-rates can improve candidate selection. (2) Even interactions at the extremes of kinetic behavior, for example, with very slow off-rates, can be detected and differentiated with confidence.

Scope of performance

Analyze up to 384 samples in unattended runs

Biacore T200 supports the use of 96- and 384-well microplates and vials. The use of all four flow cells allows four interactions to be simultaneously monitored. The sample compartment of Biacore T200 can be cooled down to 4°C, enabling the analysis of sensitive samples in unattended runs of up to 48 h.

Predict in vivo behavior by studying interactions at physiological temperatures

By providing reliable data at physiological temperatures (Fig 6), Biacore T200 enables the *in vivo* behavior of therapeutics to be more confidently predicted. An integrated buffer degasser prevents the formation of air bubbles at elevated temperatures, helping to ensure higher quality data. The integrated degasser also eliminates the need for buffer degassing before the run.

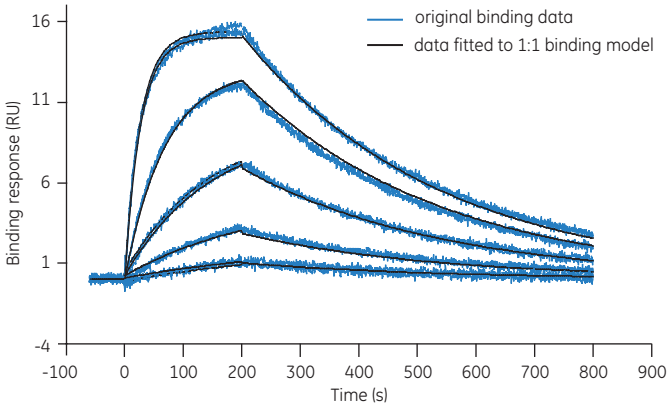

Fig 6. Biacore T200 delivers stable and reproducible data at 37°C.

Perform buffer scouting for fast assay development

With the built-in buffer selector, up to four buffers can be tested in a single run, accelerating assay development. For example, microenvironmental effects on binding properties can be studied in mechanistic and stability studies.

Recover samples for identification by mass spectrometry

Protein interaction analysis on Biacore T200 in combination with mass spectrometry provides the possibility to identify proteins on the basis of functional binding criteria. Sample recovery and digestion are supported by the software.

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

Software wizards and templates for ease-of-use

Biacore T200 software is suited to users at all levels of experience. Software wizards offer support throughout all assay steps from development to data interpretation, simplifying the entire process and reducing time-to-results (Fig 7).

For flexible assay design, ***Method Builder*** is an intuitive, powerful tool. Predefined methods may be used directly or modified for specific applications. Alternatively, users may choose to develop novel, customized methods.

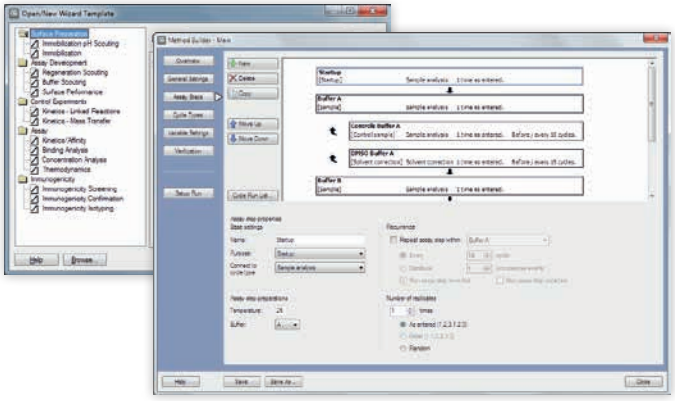

Fig 7. Easy-to-use wizards guide you through the setup of common assays while more advanced methods can be customized using ***Method Builder***.

Fast, simple kinetic analysis

Biacore T200 software offers a range of tools for confident and reliable kinetic analyses. These can be performed using a multicycle approach (using one sample concentration per cycle), or alternatively, using single-cycle kinetics. By eliminating the need for surface regeneration between injections, single-cycle kinetics simplifies analyses involving targets that are difficult to regenerate (Fig 8) and reduces assay development time.

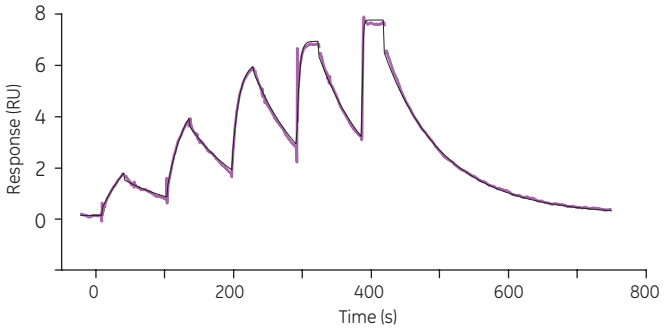

Fig 8. Using the single-cycle kinetics approach, samples are injected one after the other in the same cycle with no intervening regeneration steps. Here, a dilution series of a molecule with a relative molecular mass (M_r) of 312 was prepared at concentrations of 0.062, 0.185, 0.556, 1.667 and 5 µM, and sequentially injected over a sensor surface prepared with a protein (M_r 29 000) immobilized at 760 RU.

Efficient affinity and kinetic evaluation tools

Biacore T200 Evaluation Software enables kinetic and affinity evaluations of interactions to be performed with a few simple clicks.

- Support for both characterization and focused screening applications
- Rapid overview and qualification of data
- Flexible tools for customized data analysis

Affinity and kinetic evaluations can be performed with up to 200 concentration series in one single evaluation with data from one or several runs. A single display provides a holistic overview in a thumbnail pane while simultaneously giving details of the selected data series.

Data processing can readily be performed on individual data points, a selected subset, or all data series.

The results of the entire evaluation are compiled in a sortable and customizable table format. Resulting affinities are displayed in a K_D plot while kinetic parameters are visualized in an On-off rate map (Fig 9). This flexible setup enables simple and powerful processing of data, streamlining the evaluation process and getting to final results faster.

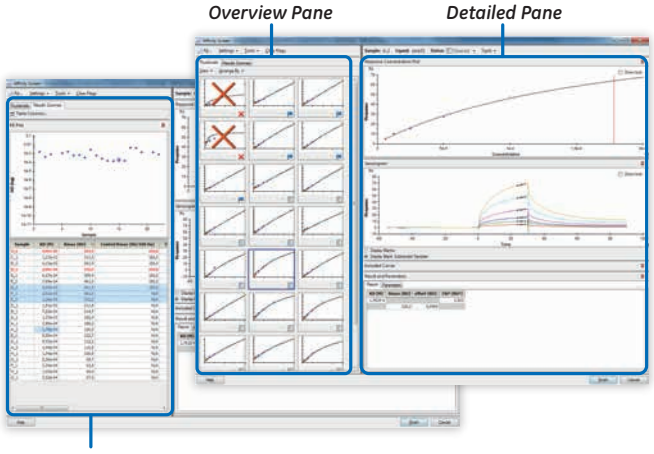

Fig 9. Good overview, flexible analysis, and comprehensive result summaries enable kinetic and affinity evaluations to be performed faster.

Simple QC tools for easy assessment of kinetic data

Automated QC tools analyze data fitting quality for the magnitude of kinetic constants, parameter uniqueness, bulk refractive index, and residuals, enabling the user to interpret results with confidence.

Visualize and make the right selections using the *Result Plot*

Result plot provides tools to plot the sample response versus a selection of variables. Up to 5000 single-concentration samples from multiple runs can be co-evaluated in a single ***Result Plot*** (Fig 10). Co-evaluation of several runs provides a full overview of the data and improves the quality of results by enabling the same adjustments and normalizations to be applied. Repetitive operations are removed saving time and reducing the risk of user-mediated errors. Selections in the data set can be performed by ranking or applying an automated control-based cutoff.

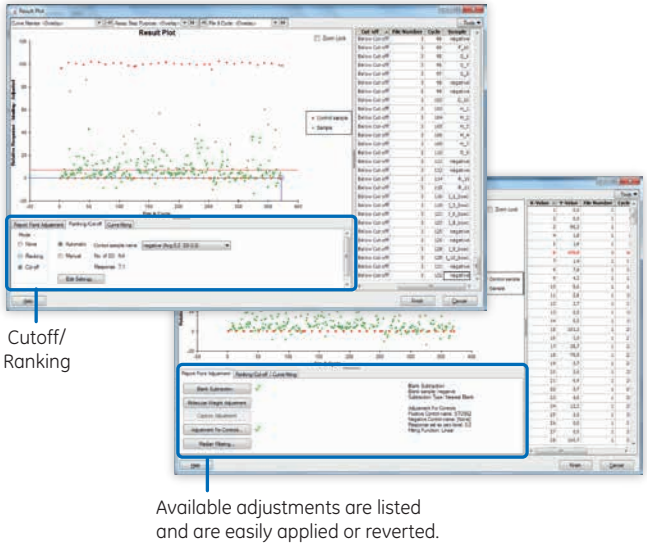

Fig 10. Using ***Result Plot***, up to 5000 samples from several runs can be co-evaluated enabling easy selection of the samples of interest.

Comparability assessment with *Sensorgram Comparison*

Comparison of binding data is an important step in late-stage development and QC of biotherapeutics. It is essential to understand and monitor any possible effect on target binding activity upon product and process changes to ensure drug safety and efficacy. Kinetic and report point analysis is typically used but becomes challenging or even insufficient when the binding data is more complex, as is the case with new generation biologics such as Fc fusion proteins, bispecific antibodies, and antibody drug candidates.

Biacore T200 Software makes comparability assessment easy by objectively comparing complete binding profiles of samples against that of a reference standard. The ***Sensorgram Comparison*** tool enables quantitation of target binding similarities using the ***Similarity Score*** for both complex and simple binding data. Up to 100 data sets from different runs can be appended enabling rapid co-evaluation of, for example, historical product batches. Co-evaluation and co-editing improves the quality of results, reduces the risk for user-mediated errors, and saves time by applying the same adjustments and normalizations of the data. Results, including the ***Similarity Score***, are summarized in a ***Results Summary Table*** and clearly displayed in a ***Scatter Graph*** or sortable ***Bar Graph*** (Fig 11).

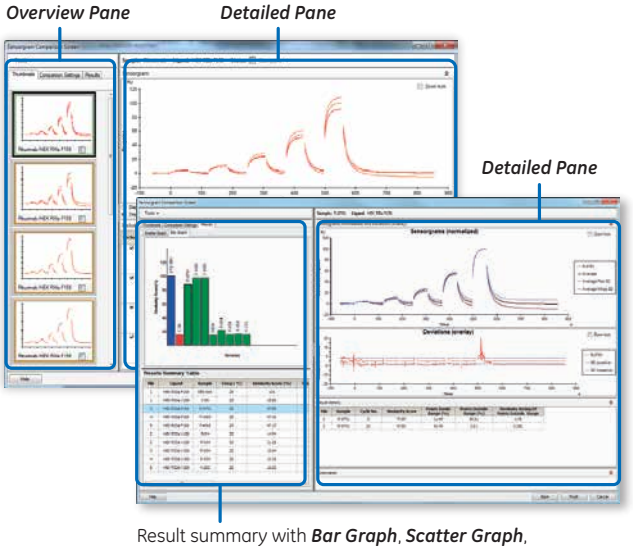

Fig 11. The ***Sensorgram Comparison*** tool makes comparability assessment easy by objectively comparing complete binding profiles of samples against that of a reference standard.

Confident concentration analysis – with or without standards

Software-supported direct binding and inhibition assays on Biacore T200 enable measurement of active concentration, and not just total protein. The precision and automation of the system generates highly reproducible data with CV typically below 5%.

- Generate highly reliable data from run to run by intrapolation of repeated calibration curves
- Ensure rigorous QC by inclusion of control samples

Calibration-free concentration analysis

Calibration-free concentration analysis (CFCA) does not require a standard curve, but relies on measured changes in binding rates at varying flow rates, under conditions where transport to the sensor surface of binding partners in solution is limited by diffusion.

This method is of great value during the development of therapeutic and immunotherapeutic protens as well as for predicting potency, since here it is important to know true concentration in relation to specific binding activity.

- Use CFCA where no satisfactory calibration standard is available
- Use as a check on the validity of the specified concentration in standards

Thermodynamic analysis for additional information about reaction mechanisms

Derive transition state thermodynamics from kinetic rate constants

Fully understanding molecular recognition by being able to predict binding energetics from the three-dimensional structure of protein complexes by thermodynamic analysis may well provide the basis for structure-based molecular design of drugs and engineered antibodies.

Dedicated software wizards, built-in buffer degassing, and temperature control in Biacore T200 make transition state thermodynamics easier than ever. Integration of rate constants measured across several temperatures into thermodynamic equations allows thermodynamic characterization of the transition state, revealing the forces driving the interaction (Fig 12).

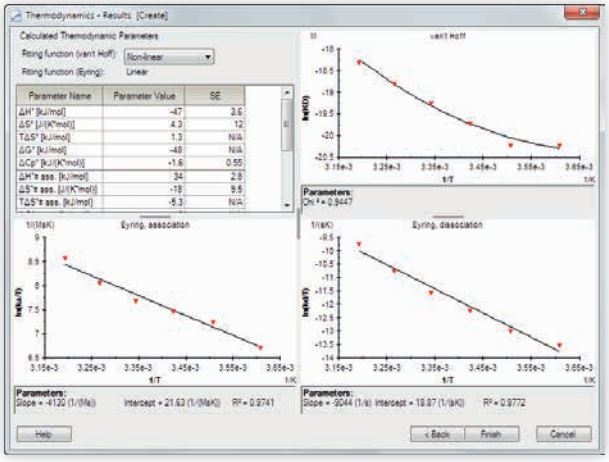

Fig 12. Automatic generation of Eyring and van't Hoff plots from kinetic data for calculation of thermodynamic parameters.

Dedicated support for immunogenicity testing

Biacore T200 software provides dedicated tools for immunogenicity testing for confident detection and characterization of anti-drug antibodies (ADA) during preclinical and clinical development. Biacore T200 may be integrated at several points throughout the entire immunogenicity workflow at screening, confirmation (elimination of false positives), and characterization (isotype determination, epitope mapping, neutralization capacity).

- Detect low affinity ADA, easily missed in endpoint assays due to losses during washing steps (Fig 13)
- Detect ADA even in the presence of drug, avoiding false negative results
- Comprehensive characterization of ADA with dedicated software tools

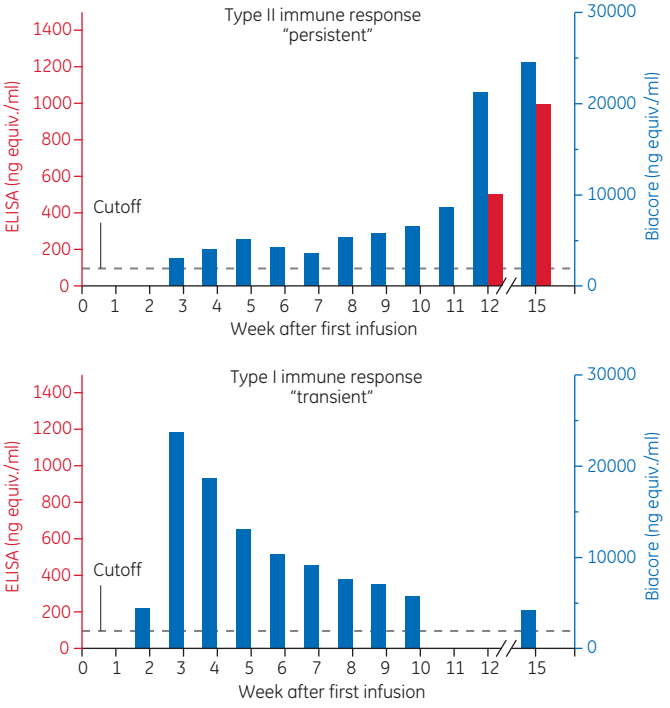

Fig 13. A comparison of data from a bridging ELISA assay and a Biacore assay in a Phase I study for a therapeutic antibody. Positive samples were detected much earlier in the Biacore assay and led to the implementation of Biacore as the preferred choice for immunogenity screening. Data courtesy of Dr. Ulrich Kunz, Boehringer Ingelheim Pharma GmbH & Co. KG

Work in GxP-regulated environments

Optional software for GxP compliance

An optional GxP Package allows Biacore T200 to integrate seamlessly into GxP-regulated workflows. The package provides validated software supporting GLP/GCP/GMP and 21 CFR Part 11 compliance, and includes validation support. For full validation support during the lifetime of the system, the package can be supplemented with GE Healthcare Validation GxP Services.

Features in the GxP Package 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 assay run and evaluation settings to be locked together in routine assays
- Audit trail – tracks record modifications and maintains complete version histories for published procedures
- Change Control Procedures (CCP), performed as required following hardware and software changes

Data can be exported both manually and automatically in Microsoft® Excel® (XLS) format as well as Extended Markup Language (XML) format. The software has been developed in accordance with an accepted development model to ensure adequate validation.

Biacore T200 specifications

Technical specifications and characteristics

Detection technology	Surface Plasmon Resonance (SPR) biosensor
Information provided	Kinetic and affinity data (K_D, k_a, and k_d), specificity, selectivity, concentration, and thermodynamic data
Data presentation	Result tables, result plots, and real-time monitoring of sensorgrams
Analysis time per cycle	Typically 2 to 15 min
Automation	48 h unattended operation
Sample type	LMW 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, and serum)
Required sample volume	Injection volume plus 20 to 50 µl (application dependent)
Injection volume	2 to 350 µl
Flow rate range	From 1 to 100 µl/min
Flow cell volume	0.06 µl
Flow cell height	40 µm
Sample/reagent capacity	1 × 96- or 384-well microplate and up to 33 reagent vials
Analysis temperature range	4°C to 45°C (maximum 20°C below ambient temperature)
Sample storage	4°C to 45°C (maximum 15°C below ambient temperature)
Sample refractive index range	1.33 to 1.40
Buffer selector	Automatic switching between 4 buffers
In-line reference subtraction	Automatic

Minimum computer requirements

3.0 GHz processor

RAM > 1 GB free

CD-ROM drive

Hard disk drive > 2 GB free

Graphics resolution at least 1280 × 1024

Typical working ranges

Association rate constant (k_a)	Proteins: 10^3 to 3 x 10^9 $M^{-1}s^{-1}$ LMW molecules: 10^3 to 5 x 10^7 $M^{-1}s^{-1}$
Dissociation rate constant (k_d)	10^{-5} to 1 s^{-1}
Sample concentration	≥ 10 pM
Molecular weight detection	No lower limit for organic molecules
Number of flow cells	4
Baseline noise	Typically < 0.03 RU (RMS)
Baseline drift	Typically < 0.3 RU/min
Recovery specifications	1.5 µl analyte recovery volume
Immobilized interactant consumption	Typically 0.03 to 3 µg/flow cell
Dimensions (W × H × D)	600 × 615 × 680 mm
Net weight total	60 kg
Mains requirements	Processing Unit Autorange 100 to 240 VAC (± 10%), 50 to 60 Hz, Class 1 equipment (protective earthing)
Power consumption	Processing Unit: max 6.3 A (at 100 VAC)

On-site requirements: Contact your local GE Healthcare representative for the latest information regarding on-site requirements.

Data handling and storage

PC operating systems	Windows® XP Professional SP3 Windows 7 Professional SP1, 64-bit Windows 8 Professional, 64-bit
Interfacing	Possibilities for import of sample data and export of results (e.g., to and from LIMS)

Ordering information

Processing unit*	Code number
Biacore T200	28-9750-01

* Includes a processing unit, control and evaluation software, and Windows operating system.

Desktop package	
Computer, screen, and printer - 110 V	28-9227-26
Computer, screen, and printer - 220 V	28-9227-25

Optional packages†	
Biacore T200 GxP Package	28-9779-54
GE Healthcare Validation GxP Services	BR-2001-06

† For more information, contact your local GE Healthcare representative.

Related literature	Code number
Data file: Biacore T200 Upgrade Kit	28-9794-14
Application note: Biacore comparability tool for quantitating binding similarities in IgG Fcγ receptor analysis	29-1519-21
White paper: Biacore concentration and ligand binding analyses in late stage development and quality control of biotherapeutics	29-1480-54
White paper: Outstanding sensitivity for confident label-free interaction analysis	28-9794-20
Brochure: When quality comes first. Cross new frontiers in label-free interaction analysis with Biacore T200	28-9794-12
Brochure: Getting the best out of your Biacore system	28-9924-38

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