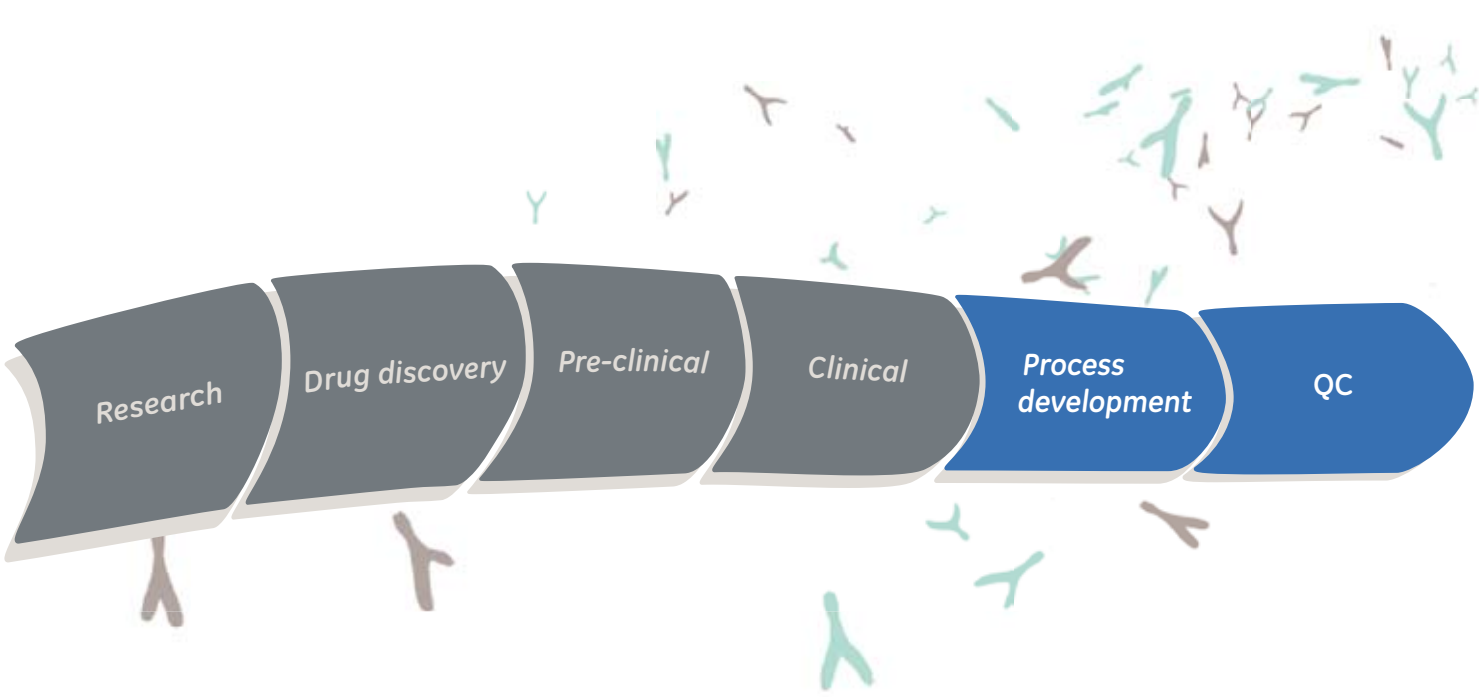
GE Healthcare

Biacore

Using label-free Biacore™ assays to improve process development and quality control



Label-free interaction analysis - from biotherapeutic discovery to manufacturing

Biotherapeutics, such as mAbs and recombinant proteins are gaining in importance due to factors such as high efficacy and relatively rapid discovery processes.

Thorough characterization of biotherapeutics is an essential aspect of the discovery & development process. For example, efficient selection, optimization and characterization of therapeutic antibodies is essential throughout development. The manufacturing and purification of proteins via large-scale cell culture (*e.g.* CHO cells) requires monitoring for potential contamination by host cell proteins. Such issues, combined with increasing demands from regulatory bodies for more frequent and extensive safety and efficacy studies places higher demands on cost efficient analytical tools in the development of biotherapeutics, from discovery to manufacturing.

Biacore™ systems from GE Healthcare deliver comprehensive characterization of biotherapeutic-target binding events, as well as rapid and reproducible determination of protein concentrations.

What value can Biacore systems add in the upstream and downstream processing?

Effective process development work can reduce costs at the clinical production scale, although the process development itself must be carried out in a time-efficient and economically controlled manner. Consideration must also be given to regulatory requirements, e.g. the concentration of recombinant proteins or mAbs derived from cell culture must be monitored throughout the production process using validated assays.

Biacore systems offer open and automated label-free assays that provide highly reproducible and reliable concentration and kinetic measurement and profiling data. Such data helps to optimize cell line and purification process and to produce high quality biotherapeutics products.

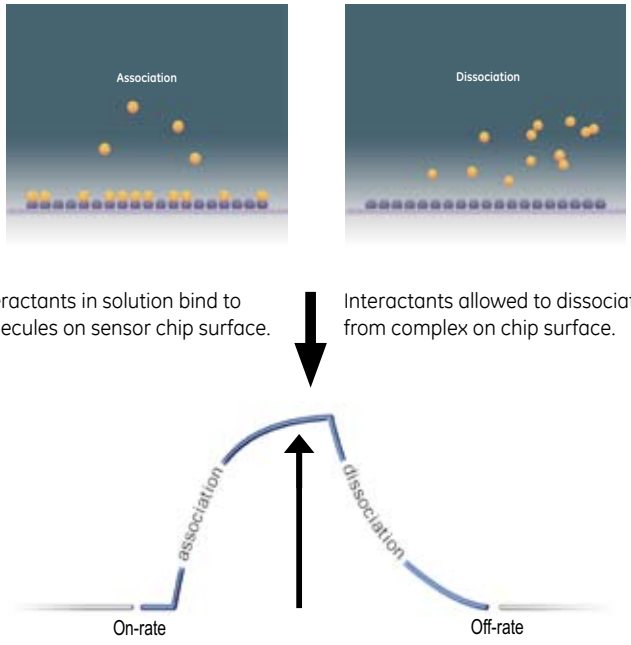
What are the advantages of using Biacore systems over alternative techniques?

- Biacore systems are designed for working in GLP/GMP/GCP environment and 21 CFR part 11 compliance.
- Measures specific binding activity not only concentration, without the need of a calibration curve.
- Provides in built quality control of quantitative data.
- Kinetic data and profiling supports drug potency and stability studies.
- Automated and label-free analysis system.
- Rapid and easy assay development and assay transfer.

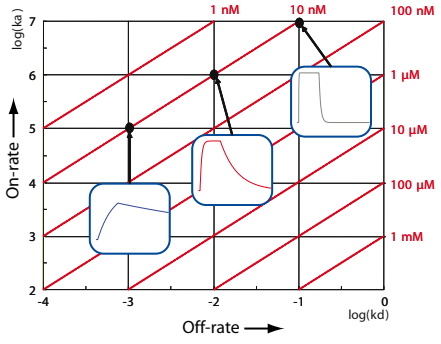
Are the binding kinetics of biotherapeutics important?

Kinetic characteristics determine how a drug interacts with its target *in vivo*. Specific kinetic properties of biotherapeutics may affect distinct aspects of drug performance e.g. through changes in process and production conditions. Monitoring of drug product by use of binding profiles is an increasingly common component of batch release testing.

- on-rates important for dosing issues – concentration dependent
- off-rates dictate target residence time of drugs – a critical property with multiple consequences



Real-time plots of binding response (the sensorgram) provides information on different kinetic profiles (*i.e.* on & off rates), affinities and protein concentration based on specific binding characteristics.



Schematic on-off rate map. Three proteins sharing the same 10 nM affinity diagonal have on- and off-rates differing by several orders of magnitude. Simulated sensorgrams show the corresponding binding profiles (boxed insets). Binding profiles can be used to monitor batch to batch variations.

Why use Biacore systems for concentration analysis?

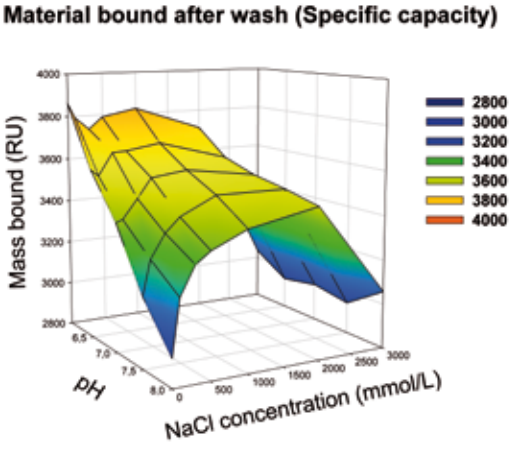
The principles of concentration measurement with Biacore systems are largely similar to established methods such as ELISA, except that in Biacore assays the entire interaction is monitored and quantified, allowing rate-based as well as end-point measurements. The advantage of using Biacore assays for concentration determination are;

- Real time detection - no need for secondary-or labelling reagents
- In-built quality control of each assay step
- The detection method allows complex samples such as feed and supernatant matrix to be analyzed

How have Biacore systems been used in UPSTREAM and DOWNSTREAM processing?

Optimization of purification conditions for a generic mAb purification process.

Antibodies are diverse, which needs to be taken into account when establishing the purication process. Biacore systems provide an excellent tool for identification, selection and optimization of capture protein conditions and the most effective elution conditions. Below is an example of IgG in different purification conditions, where the amount of IgG was measured after different purification steps.



Graphical presentation: selection of the best generic conditions for capturing a diverse range of IgGs. Data provided courtesy of Novo Nordisk AS.

Concentration determination of IgG in complex feed, supernatant matrix as well as buffer composition

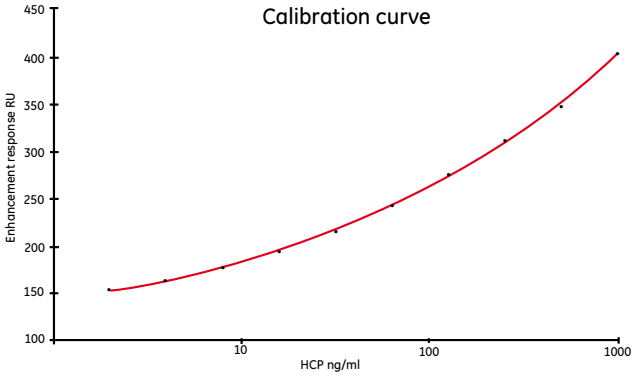
Fast and reproducible quantification of IgG was performed within 5 minutes or less. The automated analysis using Biacore systems provides a wider measuring range than ELISA and an inter-assay variability of less than 3 CV %

	Human IgG ELISA			Biacore system		
Sample	Titer	CV	Recovery	Titer	CV	Recovery
Sample 1 97 µg/ml	90 µg/ml	6-8%	70-110%	86 µg/ml	2.60%	87-103%
Sample 2 965 µg/ml	770 µg/ml	15%	70-110%	769 µg/ml	1.50%	87-103%
Measure-ment range	2.5-20 ng/ml			100-12800 ng/ml		

Comparison between a human IgG ELISA and Biacore analysis. The samples were diluted to fit calibration range. Data provided courtesy of Merck Serono, France.

Concentration of impurities

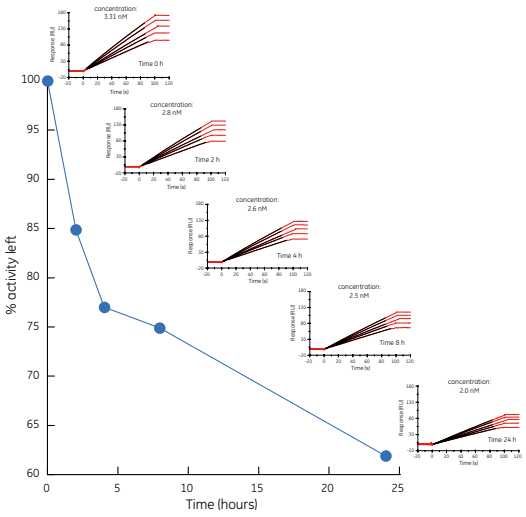
Host-cell–derived biological molecules may have toxic activity. Removal of host cell proteins (HCP) is therefore desirable to prevent potential adverse effects, and the presence of any remaining HCP needs to be checked for after the purification steps. Biacore systems are flexible in terms of assay design, allowing direct or indirect binding formats to suit experimental requirements.



Calibration curve of Host Cell Proteins from CHO cells.

Calibration free concentration analysis (CFCA)

Knowing the accurate concentration of a drug in relation to specific binding activity is invaluable information and can be used to assess product activity, stability, batch-to-batch consistency of therapeutic drug. Biacore T100 provides calibration-free concentration analysis to enable quantitative analysis when no standard is available, or where an un reliable standard would be the only alternative.



Stability study based on spedific binding activity, of target protein during exposure to stress in the abscence of a reliable standard. The concentration of target protein was measured α-chain of IgE-receptor. In less than 8 hours, loss of activity was seen due to decrease in specific binding activity. Data provided courtesey of Resistentia Pharmaceuticals.

How have Biacore systems been used in MANUFACTURING and QC?

During in-process and quality control, validated assays monitor the biological product through multiple stages of expression and purification. The high quality, real-time analysis provided by Biacore systems enables rapid, reliable quality control monitoring with minimal assay development time. For example, Biacore assays can be used in place of ELISA, reducing assay times and shortening the overal purification process significantly. Inclusion of kinetic analysis into QC monitoring provides a unique possibility to get detailed binding data that is highly relevant for clinical function of the biological product. Batch differences can easily be identified by monitoring binding interaction profile in comparison to a master for every batch produced.

The comprehensive data generated using Biacore systems, support decisions to early terminate a production batch or to adjust the culturing conditions.

Improve upsteam and downstream processing

Using Biacore systems in process development and manufacturing will enable you to:

- Reduce development time when optimizing cell line and purification conditions
- Quantitative data without the need of standard.
- Improve productivity and data quality.
- Improve consistency of product quality.
- Easily transfer assays developed in-house, without compromising established performance standards, to an outsourced partner.

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imagination at work

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