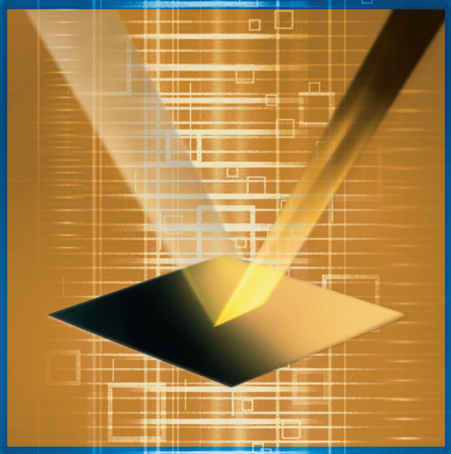
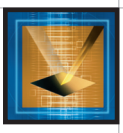
An introduction to Biacore's SPR technology



BIACORE

Biacore's SPR technology – at the core of scientific research



As the pioneer and world leader in the development of Surface Plasmon Resonance (SPR) technology, Biacore has revolutionized scientific research by enabling the real-time detection and monitoring of biomolecular binding events – so paving the way to a better understanding of biochemical mechanisms.

In addition to identifying binding partners to target molecules, Biacore provides quantitative information on:

- Specificity – how specific is the binding between two molecules?
- Concentration – how much of a given molecule is present and active?
- Kinetics – what is the rate of association / dissociation?
- Affinity – how strong is the binding?

Functional analysis of molecular interactions

Biacore's SPR technology has been designed to investigate the functional nature of binding events. Its reliability and success is built on Biacore's unique expertise in three cornerstone technologies:

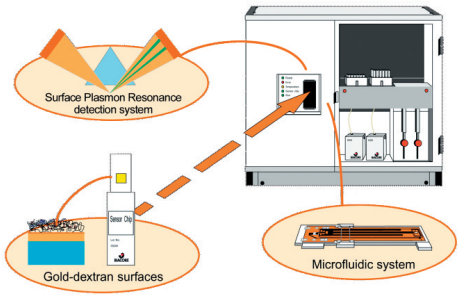


FIGURE 1: Biacore's three cornerstone technologies

1. Sensor Chip technology

The Biacore sensor chip is at the heart of the technology. Quantitative measurements of the binding interaction between one or more molecules are dependent on the immobilization of a target molecule to the sensor chip surface.

Binding partners to the target can be captured from a complex mixture, in most cases, without prior purification (for example, clinical material, cell culture media) as they pass over the chip. Interactions between proteins, nucleic acids, lipids, carbohydrates and even whole cells can be studied.

The sensor chip consists of a glass surface, coated with a thin layer of gold. This forms the basis for a range of specialized surfaces designed to optimize the binding of a variety of molecules.

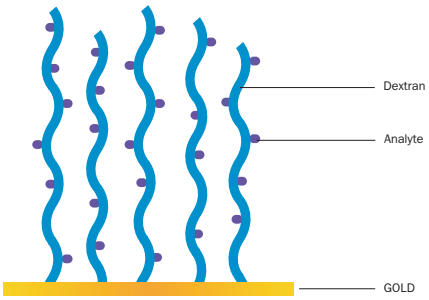


FIGURE 2: The sensor chip surface

In the most widely used sensor chip, the gold surface is modified with a carboxymethylated dextran layer (see fig 2). This dextran hydrogel layer forms a hydrophilic environment for attached biomolecules, preserving them in a non-denatured state. A range of other derivatized surfaces is also available to enable various immobilization chemistries. Whatever the molecule of interest, there is a Biacore sensor chip to satisfy research needs:

- **Sensor Chip CM5** – for applications from basic research to quality control
- **Sensor Chip SA** – for capture of biotinylated peptides, proteins and DNA
- **Sensor Chip NTA** – for capture of ligands via metal chelation
- **Sensor Chip HPA** – for membrane biochemistry and the study of membrane associated receptors in a near native environment
- **Pioneer Chips** – as tools for users' novel applications in terms of chemistry or binding specificity.

Biacore's expertise in surface chemistry means that whatever the choice, Biacore sensor chips provide reproducible results, stable baselines, high chemical stability and low non-specific binding. Stability is such that sensor chip surfaces can be regenerated for many cycles depending on the nature of the immobilized ligands – 100 is average but as many as 400 are possible. They can also withstand high salt concentrations, extremes of pH and organic solvents. Importantly, Biacore sensor chips are easily interchangeable – providing great flexibility in the research environment.

2. Microfluidics

Biacore has developed a flexible microfluidics system for its SPR technology based around the Integrated micro Fluidics Cartridges (IFC), which are specific to defined instrument series (see fig 3). All IFCs allow analyte to pass over the sensor surface in a continuous, pulse-free and controlled flow – maintaining constant analyte concentrations at the sensor chip surface.

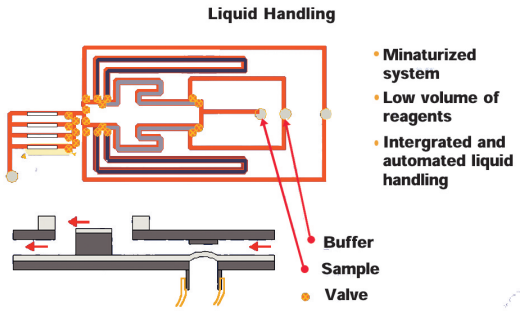


FIGURE 3: The IFC currently used in the Biacore®3000

The benefits of Biacore's microfluidics system are:

- low sample consumption
- the absence of an air-solution interface where samples can evaporate and proteins can be denatured
- the concentration of free analyte is constant and therefore known at all times
- sample dispersion in the IFC is kept to a minimum enabling accurate control of sample contact times
- no washing steps are needed to replace the sample with buffer
- a range of surface ligand concentrations and contact times can be analysed in one experiment – improving kinetic and concentration analysis
- accurate temperature stability is ensured throughout the analysis
- depending on the system used, up to four channels can be measured simultaneously – allowing comparison with a blank and automatic reference subtraction, and saving time when screening large numbers of samples
- channels can be used in different configurations, so that more than one experiment can be run at the same time.

3. Surface Plasmon Resonance detection

The gold layer in the sensor chip creates the physical conditions required for Surface Plasmon Resonance (SPR). Essentially, SPR detects changes in mass in the aqueous layer close to the sensor chip surface by measuring changes in refractive index.

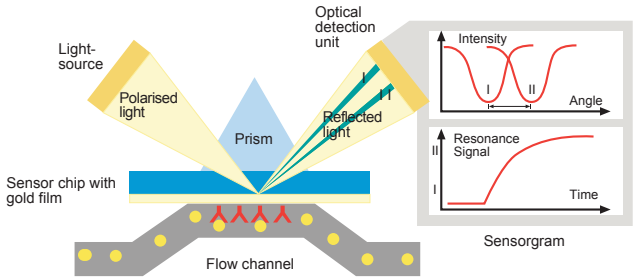


FIGURE 4. Illustration of detector with sensor chip and IFC

When molecules in the test solution bind to a target molecule the mass increases, when they dissociate the mass falls. This simple principle forms the basis of the sensorgram – a continuous, real-time monitoring of the association and dissociation of the interacting molecules (see fig 5). The sensorgram provides quantitative information in real-time on specificity of binding, active concentration of molecule in a sample, kinetics and affinity. Molecules as small as 100 Da can be studied.

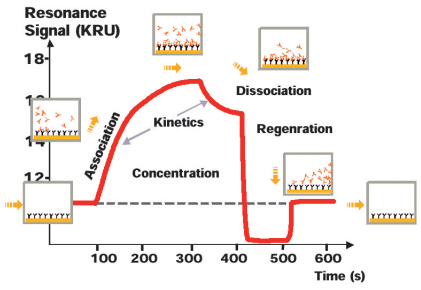


FIGURE 5. The sensorgram

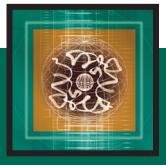
Studies can be carried out in colored or opaque environments, and there is no need to label molecules with fluorescent or radioactive tags – so avoiding the possibility that labels may compromise activity. Molecules can be studied in their native state to provide results that reflect *in vivo* activity.

Powerful software makes experimental design easy

Menu-driven software guides users through the experimental set-up. Researchers can perform simulations of experimental conditions to determine minimum concentrations needed to see binding - as well as exploit "wizards", on certain instruments, for rapid experimental planning and data analysis.

Fundamental across a diverse range of research areas

Biomolecular binding interactions are fundamental to research, not only within life sciences, but also for drug discovery and development, and food analysis. Biacore's SPR technology, a tool designed for the functional analysis of biomolecular binding events, is being used in laboratories around the world to:

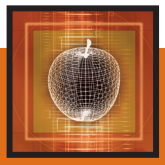


Life Science Research Applications

- Identify DNA damage – mutations, DNA adducts etc
- Determine expression levels of selected proteins under different conditions, for example, health, disease, drug treatment
- Identify binding partners to any target molecule – ligand fishing
- Elucidate the function of novel proteins in proteomics research
- Find antagonists to critical binding events
- Investigate the binding properties of protein isoforms
- Identify disease markers in clinical samples
- Optimize therapeutic antibodies
- Unravel the intricate molecular interactions involved in cell signaling pathways
- Evaluate ion selectivity in signaling pathways
- Determine the nature of protein complexes and their function
- Study membrane associated molecules in near native environments

Drug Discovery and Development Applications

- Proteomics – target identification and ligand fishing
- Target and assay validation for High Throughput Screening (HTS)
- 'Hit' to lead characterization – rapid affinity ranking and detailed kinetics of interaction for small molecules binding to target proteins
- Lead optimization through interaction kinetic based QSAR (Quantitative Structure Activity Relationship)
- Early ADME (Absorption, Distribution, Metabolism, Excretion) – rapid *in vitro* assays for defined ADME parameters, such as protein binding studies
- Clinical trials – stratification of patient response to therapeutics
- Production and manufacturing – process and batch QC of therapeutic antibodies and proteins



Food Analysis Applications

- Quality control - Rapid and reliable vitamin analysis in health and nutrition food manufacturing
- Food Safety - On-site screening for veterinary drug residues such as antibiotics and hormones in dairy and meat production plants
- Food Safety - Optimization of screening assays for monitoring hazardous natural toxins in food and feed.
- R&D - Assay construction for the identification of hormone disrupters and to investigate their mechanism of action.
- Process control - Monitoring of specific analytes in food and beverage production plants

Sample analysis using Biacore's SPR technology is rapid – some assays can take as little as a few minutes. Fully automated systems can perform unattended runs for up to 384 samples – so making Biacore's SPR technology a perfect tool for busy laboratories.

Ideal for both research and routine use, Biacore SPR is cited by over 2000 scientific, peer-reviewed papers from leading academic groups, national research centers and major pharmaceutical and biotechnology companies worldwide.

For further information see the Biacore website at: www.biacore.com

References:

Real-Time Analysis of Biomolecular Interactions:
Applications of Biacore.
Editors Nagata, K. and Handa, H. Springer-Verlag,
Tokyo (2000)

R. L. Rich and D. G. Myszka.

Advances in surface plasmon resonance biosensor analysis

Curr Opin Biotechnol 11: 54-61 (2000)

M. Malmqvist.

BIACORE: *an affinity biosensor system for characterization of biomolecular interactions.*

Biochem Soc Trans 27: 335-340 (1999)

D. G. Myszka and R. L. Rich.

Implementing surface plasmon resonance biosensors in drug discovery.

Pharmaceutical Science and Technology Today 3: 310-317 (2000)

M. Lackmann

Isolation and characterization of "orphan-RTK" ligands using an integrated biosensor approach

Methods Mol Biol 124: 335-59. (2001)

V. Z. Slepak

Application of surface plasmon resonance for analysis of protein-protein interactions in the G protein-mediated signal transduction pathway

J Mol Recognit 13: 20-26 (2000)

H. E. Indyk, E. A. Evans, M.C. Bostrom Caselunghe,
B. Persson, P. M. Finglas, D. C. Woollard, E. L. Filonzi

Determination of biotin and folate in infant formula and milk by optical biosensor-based immunoassay

Journal of AOAC International 83: 5, 1141-1148 (2000)

G.A. Baxter, M. C. O'Connor, S.A. Haughey,
S.R.H. Crooks, C.T. Elliott

Evaluation of an immunobiosensor for the on-site testing of veterinary drug residues at an abbatoir. Screening for sulfamethazine in pigs.

Analyst 124: 1315 -1318 (1999)

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