BIAcore™ Surface Plasmon Resonance

EXPLANATORY NOTE 101

A Description of the Detection Technique

BIAcore employs a unique optical phenomenon that occurs when light illuminates certain metals under specific conditions. Surface plasmon resonance (SPR) is a result of magnetic vectors of incident light resonating with the free electron clouds in specific thin metal films. This phenomenon was initially investigated by Turbadar[1] although it was the work by Otto[2] and Kretschmann and Raether[3] that brought versatility to the technique. For reviews about SPR the reader is referred to Welford[4] and Raether.[5]

To begin describing SPR, it is helpful to start with the electromagnetic phenomenon total internal reflection (TIR). TIR occurs when light (beyond the critical angle and incident on an interface of higher to lower refractive index) is totally reflected as described by Hirschfield.[6] When TIR occurs, an evanescent wave propagates away from the interface into the lower refractive index medium (see Figure 1).

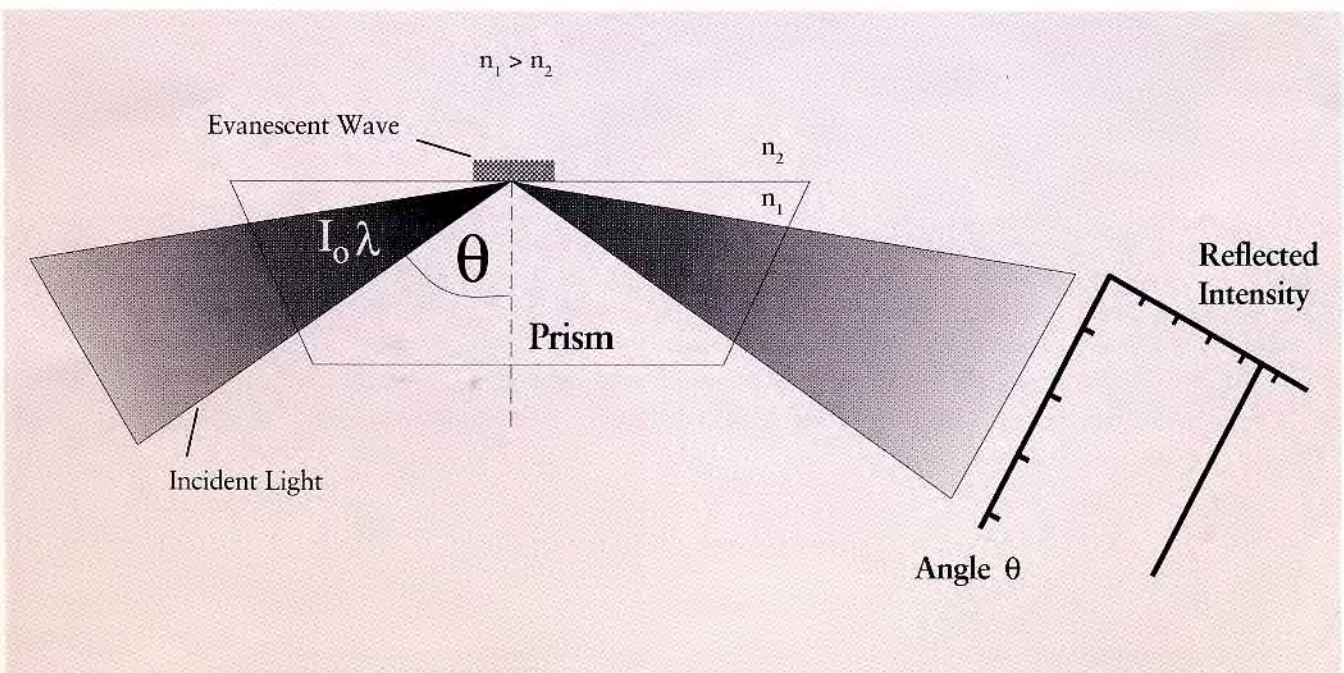

Figure 1.

If one maintains the same optical configuration but now inserts a thin metal film at the interface, a new phenomenon can occur. Some metals, e.g., gold and silver, have free electron clouds which can couple and resonate with certain angles of incident light. These electron clouds, termed plasmons by Hopfield,[7] have a collective wave vector parallel to the interface. The resonance absorbs energy from the incident light and a characteristic drop in reflected light intensity is demonstrated (see Figure 2). This is a well known phenomenon in integrated optics as described by Tien.[8] The phase matching is very sensitive to changes in the wavelength and the thickness of the metal but if those are kept constant then only one parameter will effect the resonance. This parameter relates back to the evanescent wave that still exists, propagating from the metal interface.

The evanescent wave profile is dependent upon the refractive index of the medium it probes. By changing the refractive index at n_2 the evanescent wave is altered and that in turn changes the plasmon vector length which will require a new incident angle for resonance. The actual dielectric equations and application of this approach are discussed in detail by Kretschmann,[9] Liedberg[10] and Jönsson.[11]

To summarize the phenomenon, light incident to an interface of two refractive indices, between which a thin film of gold is placed, will resonate at a specific angle and result in an absence of reflected light. This resonance is very sensitive to refractive index changes very close to the metal surface. A change in the refractive index will require a new angle for resonance.

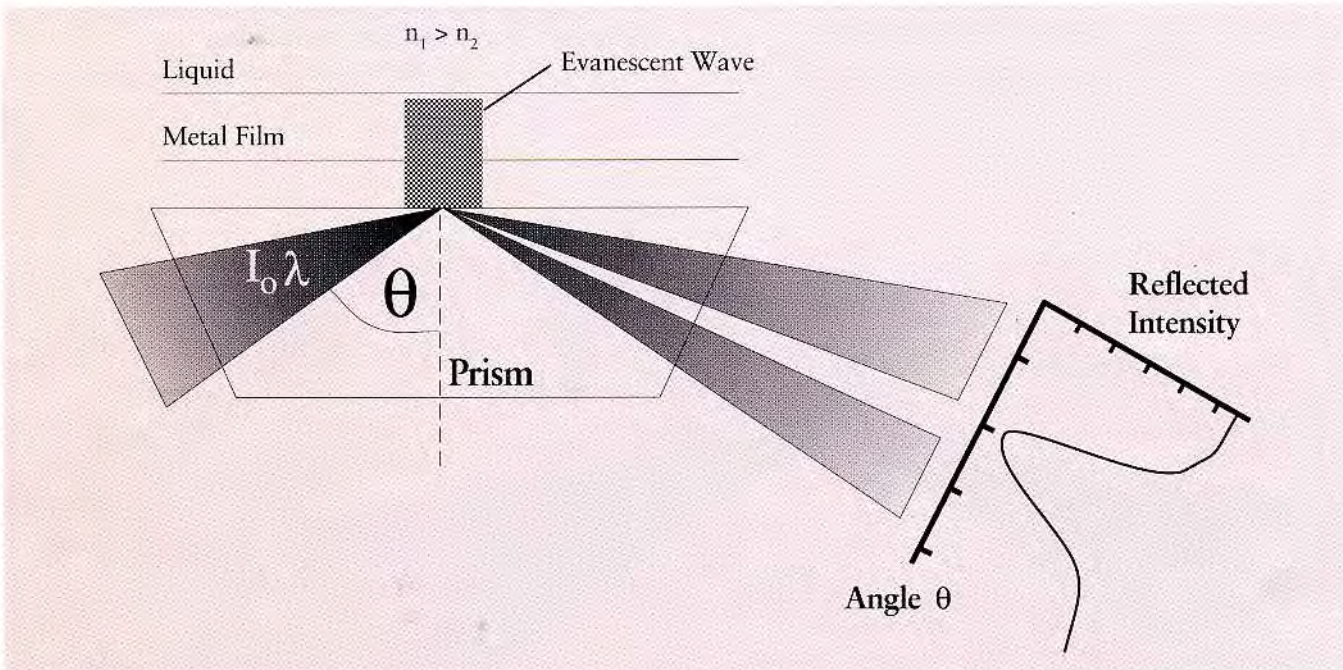

Figure 2.

Another important characteristic of this detection system is the fact described by Kovacs[12] that the evanescent wave only penetrates the lower refractive index medium, approximately a wavelength's distance from the metal surface. This allows for the measurement of refractive index changes only out to ~ 300nm after which the energy intensity is quite low since the energy follows exponential decay (see Figure 3).

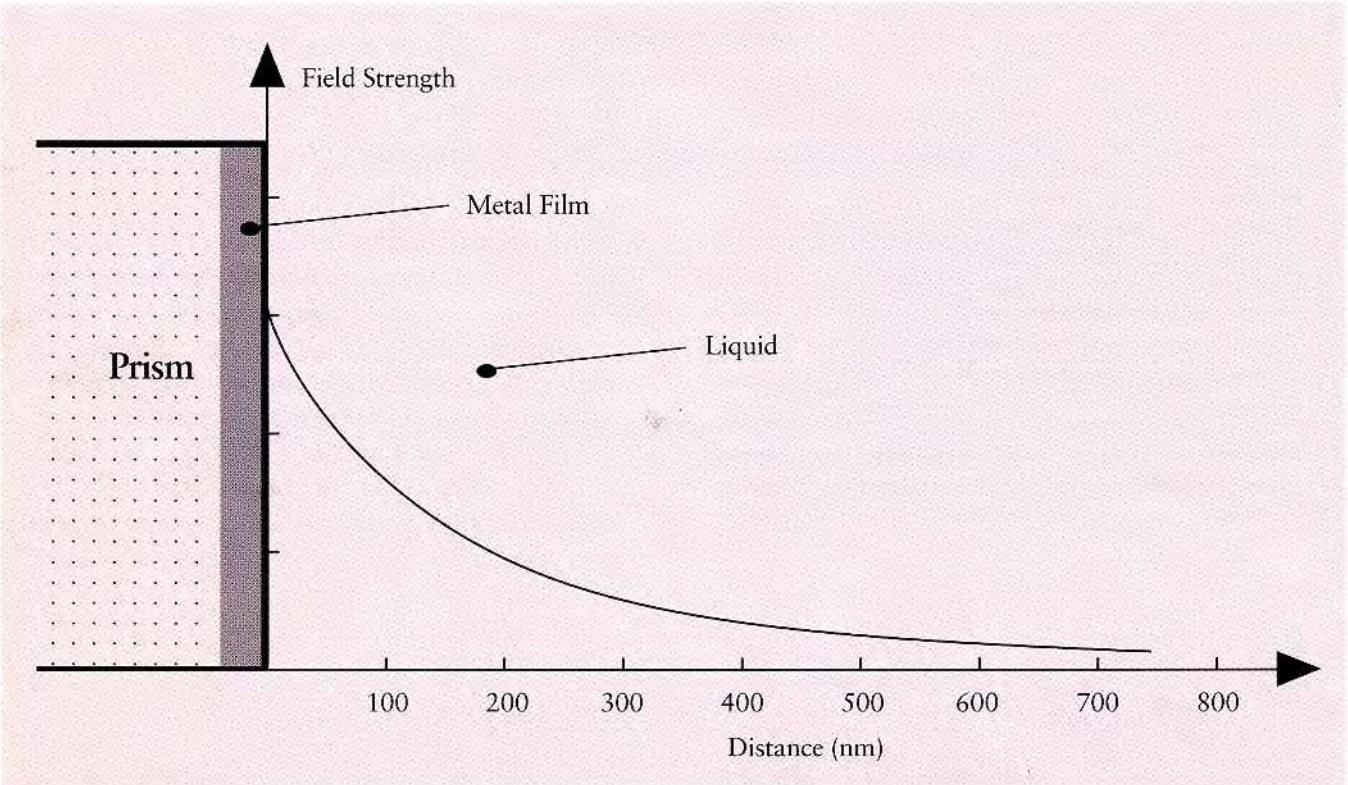

Figure 3.

Due to these characteristics it is possible to use SPR as a detection system since, under the proper geometries, SPR only probes about 300nm out from the surface. Since all proteins independent of sequence contribute the same refractive index, see Polymer Handbook[13] the possibility of a mass detector is realized. SPR sensitivity has been shown by Kooyman[14] and linear correlation between resonance angle shift and protein surface concentration has been shown by Stenberg.[15] This has made the real-time detection of mass change, without labels, possible.

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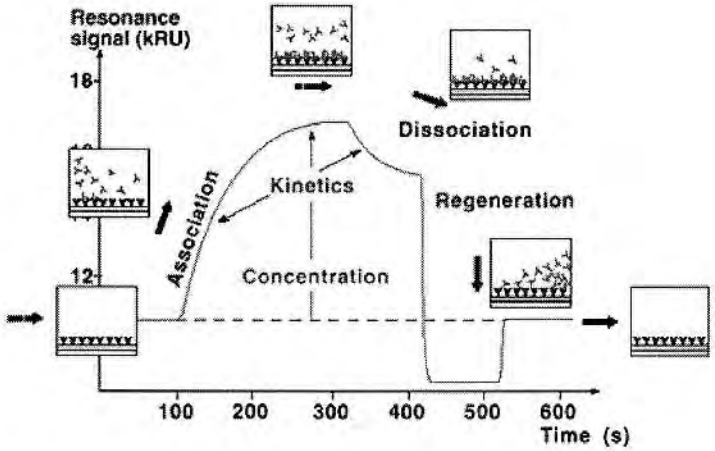
Biomolecular Interactions

BIACORE

Company Information
Biomolecular Interactions
Basics
Principles
FAQs
Key Areas
Products
Customer Service

Principles

Understanding the Sensorgram



The progress of an interaction is monitored as a sensorgram. Analyte binds to the surface-attached ligand during sample injection, resulting in an increase in signal. At the end of the injection, the sample is replaced by a continuous flow of buffer and the decrease in signal now reflects dissociation of interactant from the surface-bound complex. A response of 1000 RU corresponds to a change in surface concentration of 1 ng/mm2.

Back Next

Principles

Preparation of Sensor Surfaces

To investigate any binding event one of the interacting partners must be directly immobilized to a surface or captured by a previously immobilized capturing molecule (e.g. an antibody or a receptor).

Well defined surface chemistry allows for reproducible and flexible immobilization processes, usually taking about 20 minutes. After preparation, a surface may be used many times. Surface re-use depends mainly upon the stability of the molecule immobilized.

Carboxymethylated sensor chips enable immobilization of ligands via native - NH2 - SH2 - CHO and COOH. Other sensor chips are supplied pre-immobilized and ready for use for specific applications.

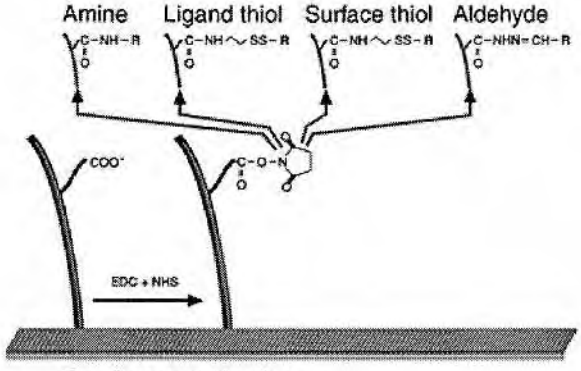
Immobilizing the Molecules

Sensor Chips

CM5 - Ligand immobilization via native - NH2 - SH2 - CHO and COOH
SA- Capture of biotinylated peptides, proteins and DNA
NTA- Capture of ligands via metal chelation
HPA - Surface modification via hydrophobic interaction

For additional sensor chips, see Pioneer products

Ligand immobilization via native - NH2 - SH2 - CHO and COOH



Covalent derivatization

Back Next

Biomolecular Interactions

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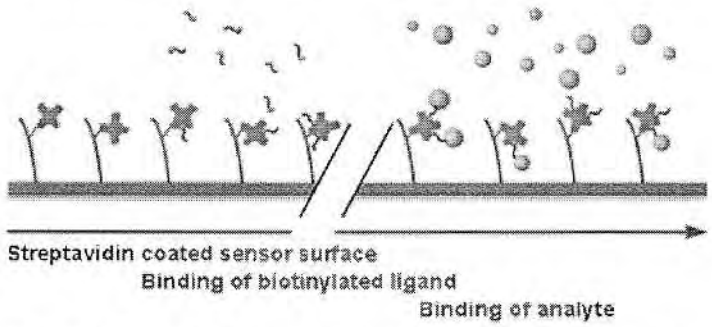
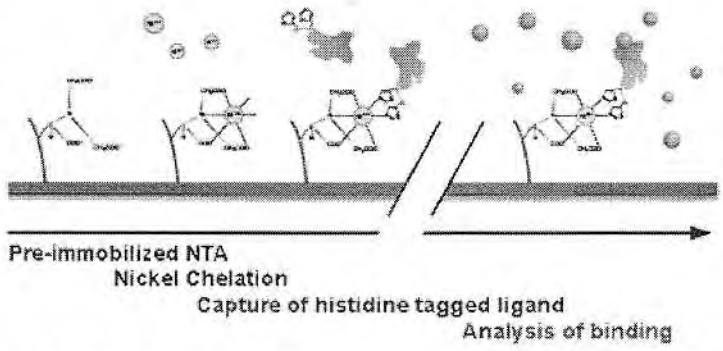
Principles

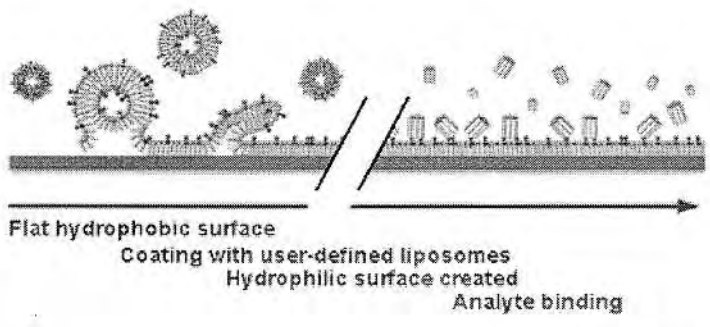
Capture of biotinylated peptides, proteins and DNA



Capture of ligands via metal chelation including His-tagged proteins



Membrane biochemistry and membrane-bound receptor studies



Back Next

Biospecific interaction analysis using biosensor technology

Magnus Malmqvist

Fundamental information that enhances our understanding of biospecific interactions can be obtained using a new analytical system based on biosensor technology. The functional characteristics of biospecific interaction, such as kinetics, affinity and binding position, are examined by label-free analysis of proteins in free solution binding to an immobilized ligand at a hydrophilic sensor surface.

INTERACTIONS between biomolecules are fundamental for life and the characterization of these interactions in terms of structure-function relationships is crucial for our understanding of biological systems. A new biosensor-based analytical system, BIAcore (Pharmacia Biosensor AB, Uppsala, Sweden), is designed for functional characterization of protein–protein, protein–DNA and ligand–receptor interactions in real time. To date, the analytical system has mostly been used for the kinetic analysis of monoclonal and recombinant antibody–antigen interactions and for biomolecular engineering and drug design.

SPR technology

The adsorption of biomolecules to an immobilized ligand on a sensor chip is measured in the same time and place as it occurs. The analytical system, BIAcore, is based on a biosensor that uses surface plasmon resonance (SPR)[1] to monitor the adsorption of biomolecules on a sensor chip. This optical technique measures changes in refractive index in the vicinity of the surface. Such changes are directly proportional to the change in adsorbed mass[2], which makes it suitable for the detection of biomolecules. The system includes a sensor chip to which the ligand can be immobilized in a 100-nm thick hydrophilic matrix composed of 2–3 per cent flexible dextran[3,4], a miniaturized fluidics cartridge for the transport of analytes and reagents to the sensor chip[5], a SPR detector, an auto injector and software for system control and evaluation of results. The system has been described in more detail in refs 6 and 7 (see Fig. 1).

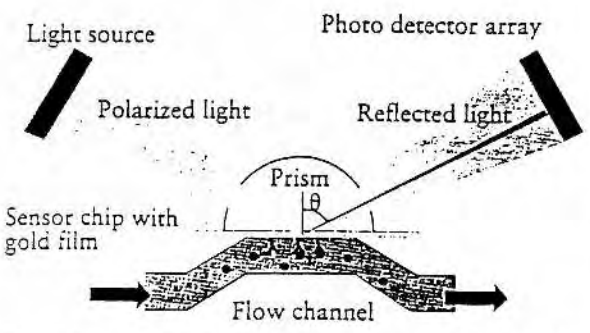


FIG. 1 The configuration of the surface plasmon resonance detector, sensor chip and integrated fluidic cartridge in the BIAcore system. The detector works with polarized light from a light-emitting diode, which is reflected in the gold film on the sensor chip and detected on a diode array. Surface plasmon resonance is observed as a decrease in light intensity for a specific angle of incidence. This angle changes with changes in the refractive index in the vicinity of the surface due to adsorption of large molecules on the immobilized ligand on the sensor chip.

Specific ligands are immobilized to the sensor chip at low concentration through biotin–avidin interaction or covalently through amine, thiol or aldehyde chemistry. This provides the user with flexibility in both selecting specificity and in the analytical procedure. The stable binding allows regeneration of the sensor surface and 50–100 analytical cycles can be performed on one and the same surface — depending on regeneration conditions. An easy to use programming environment for automating analytical procedures allows the system to run overnight and at weekends, leaving the daytime free for developing new analyses and evaluation of results. The system can also be used for standardized concentration analysis[8–10], as well as for more sophisticated analysis of kinetics and affinity.

The analysis can also be performed in tissue culture media[11] or bacterial broth[12,13] without the need for purification, in contrast to other techniques used for kinetics analysis such as stopped-flow fluorescence quenching.

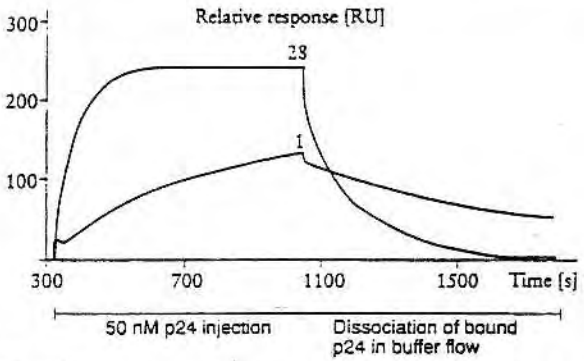


FIG. 2 Sensorgrams for adsorption of HIV-1 p24 antigen on two different mAbs. The curves illustrate the differences in association and dissociation rates and the time difference to reach equilibrium.

Applications

Kinetics. The adsorption of an analyte to a surface-immobilized ligand under constant flow can be regarded as a 'pseudo' first-order reaction as the concentration of analyte in the thin layer cell is constant. The adsorption rate can be measured using BIAcore[14–16], and from these data the association rate constants can be obtained by carrying out a series of adsorption experiments with different analyte concentrations. The logarithmic decrease in complex concentration on the surface after the sample pulse has passed can be used for the determination of dissociation rate constants.

The values obtained from kinetics and affinity measurements based on different principles and methods may differ. However, with an understanding of the fundamental differences of methods, results generated can provide useful quantitative information of interaction parameters. Using BIAcore, the analysis of nonpurified samples can be performed without labels.

Kinetic analysis of mAb binding. Kinetic information about biospecific interactions can complement affinity measurements. For example, two monoclonal antibodies (mAbs) with the same affinity for the antigen had a five-fold difference in association and dissociation rate constants[14] (see Fig. 2). Similar results were obtained for site-specific mutations of Z-fragment of protein A in their interaction with a Fc fusion protein[17]. Two mutants (L17D and K35A) both had ten-times lower affinity for Fc binding compared with the wild-type protein domain. The L17D mutant had a ten-times lower

association rate constant and K35A had a ten-times higher dissociation rate constant. These two examples show the importance of separating on and off rate constants. With a knowledge of the three-dimensional structure the information obtained can be used to relate structure to function.

Monoclonal antibodies directed against peptides from the coat protein of tobacco mosaic virus were analysed for binding both to different peptides and the protein[18]. The hydrophilic environment in the matrix is valuable here as conformational changes attributable to adsorption to plastic surfaces used in ELISA techniques are avoided. The kinetic analysis of mAb binding to a 25-amino acid peptide of the protein showed heterogeneous binding during association, in contrast with homogenous binding to a shorter peptide from the same sequence. The differences in affinity between peptides and protein could be ascribed to changes in association rate constants. Only kinetic analysis can separate these fine differences in the specificity of antibodies.

The effect of differences in peptide sequence in relation to the kinetic properties of mAb binding has been reported by Altschuh *et al.*[16], as well as the binding of mAbs to whole virus particles[19,20].

The kinetic analysis of mAbs of IgG- and IgM-type against tetanus toxoid antigens illustrates the effect of multiple binding[21], and a study of anti-hen lysozyme antibody, D1.3, and recombinant antibody fragments has been performed by the same research group[22].

Epitope mapping. Epitope mapping of human immunodeficiency virus-1 (HIV-1) core protein p24 by pairwise mapping of 30 different mAbs using the BIAcore system has been performed by Fägerstam *et al.*[11]. Both identification of peptides inhibiting the mAb binding and multiple binding of several mAbs in sequence forming a hexamolecular complex on the surface were shown (see Fig. 3). This illustrates the potential use of the system for the analysis of the formation of large functional complexes. The open structure of the surface matrix makes the formation of large complexes possible.

Recombinant antibodies. Kinetic analysis using BIAcore has been particularly useful in the rapid development of recombinant antibody technology. Affinity maturation of antibody fragments from a naive library by chain shuffling was found to be dominated by a decrease in dissociation rate constants[13]. Antibody fragments derived from a human naive library[23] characteristically had both high association and high dissociation rate constants[24]. The system has also been used as a specific detector for analytical gel filtration showing the formation of active dimers of single chain Fv antibody fragments.

New application areas

The interaction of HIV-1 gp120 and a mAb with soluble CD4 has also been analysed with BIAcore[12]. In application notes, research groups report examples of equilibrium and kinetic analysis of epidermal growth factor (EGF) and EGF receptor. The soluble receptor binding to immobilized EGF has high on and off rate constants. Other examples are the interaction of different SH2 domains with an immobilized phosphotyrosin peptide derived from platelet-derived growth factor receptor.

An application area under development is protein–DNA interactions with the immobilization of DNA through biotinylated nucleotides on streptavidin surfaces.

The technology, real-time biospecific interaction analysis, was originally developed using mAb–antigen interactions as model systems but is now rapidly being transferred to other areas of biological significance due to the general approach for label-free analysis of binding reactions. Although, to date, most scientific reports have been from academic research laboratories, the technology is now finding most applications in the pharmaceutical industry, both in research and in the quality control of recombinant proteins to be used as therapeutic agents. □

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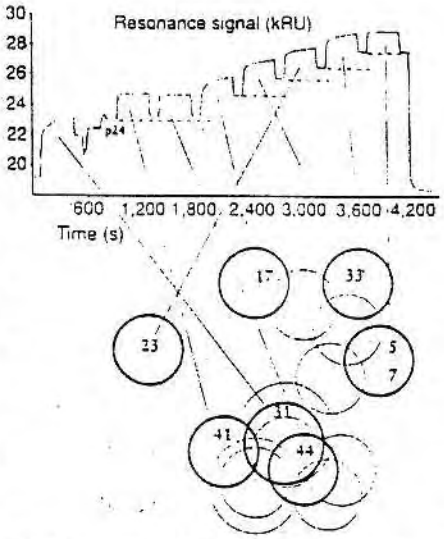

FIG. 3 Formation of a hexamolecular complex of five mAbs and HIV-1 core protein p24 on the sensor chip by adsorption of mAbs from tissue culture medium. The mAbs were selected from the epitope map illustrated in the lower part of the figure. The mAbs were sequentially injected over antigen bound to surface-immobilized mAb[11] (reprinted from ref. 11 by permission of John Wiley & Sons Ltd).

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