Label-free interaction analysis in real-time using surface plasmon resonance

Providing quantitative data on:

- **Specificity**
  To what extent does an interacting partner cross-react with other molecules?
- **Concentration**
  How much of a given molecule is present and active?
- **Kinetics**
  What are the rates of association and dissociation?
- **Affinity**
  How strong is the interaction?

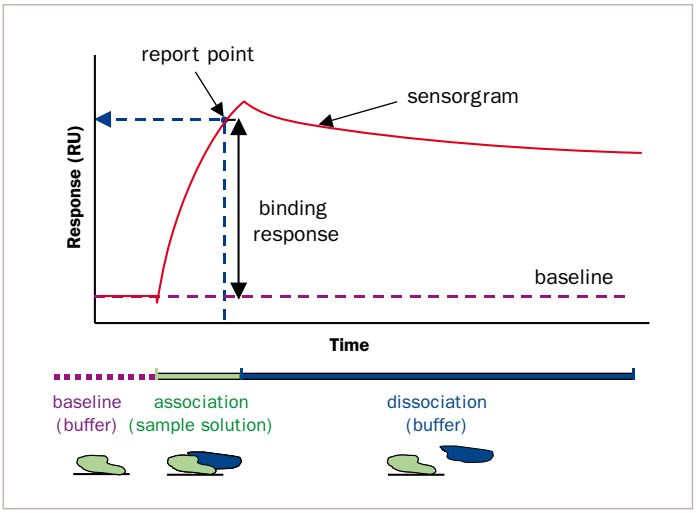

Figure 1. The sensorgram provides real-time information about an entire interaction, with binding responses measured in resonance units (RU). Binding responses at specific times during the interaction can also be selected as report points. According to the interactions under investigation, any remaining bound sample molecules may be removed in a regeneration step that prepares the surface for the next sample injection.

Introduction

Biacore™ systems monitor molecular interactions in real-time using a label-free detection method. Sample in solution is injected over a sensor surface on which potential interacting partners are immobilized, either singly in individual flow cells or as part of an array. As the injected sample interacts with the immobilized partners, the refractive index at the interface between the sensor surface and the solution alters to a degree proportional to the change in mass at the surface. The phenomenon of surface plasmon resonance (SPR) is exploited to detect these changes in real time and data are presented in a 'sensorgram', a profile of SPR response plotted against time. The sensorgram traces the association and dissociation of complexes over the entire course of an interaction, with the kinetics revealed by the shape of the binding curve (Figure 1).

Label-free detection

Using the SPR phenomenon enables interactions to be monitored without the use of labels. In order to detect changes in SPR signals, alternative configurations can be employed; prism based SPR (PB-SPR) or grating coupled SPR (GC-SPR).

Prism-based SPR

PB-SPR (Figure 2) uses polarized light to illuminate small surface areas so facilitating high sensitivity detection. As light does not penetrate the sample, interactions can be followed in colored, turbid or opaque samples. Under conditions of total internal reflection, the polarized light strikes an electrically conducting gold layer at the interface between media of different refractive indices; the glass of the sensor surface covered with a thin layer of gold (high refractive index) and a buffer (low refractive index). A wedge of polarized light, covering a range of incident angles, is directed toward the glass face of the sensor surface. Reflected light is detected within the system. An electric field intensity, known as an evanescent wave, is generated when the light strikes the glass. This evanescent wave interacts with, and is absorbed by free electron clouds in the gold layer, generating electron charge density waves called plasmons and causing a reduction in the intensity of the reflected light. The angle at which this intensity minimum occurs (the resonance angle) is a function of the refractive index, and hence molecular mass, adjacent to the gold layer on the opposing face of the sensor surface.

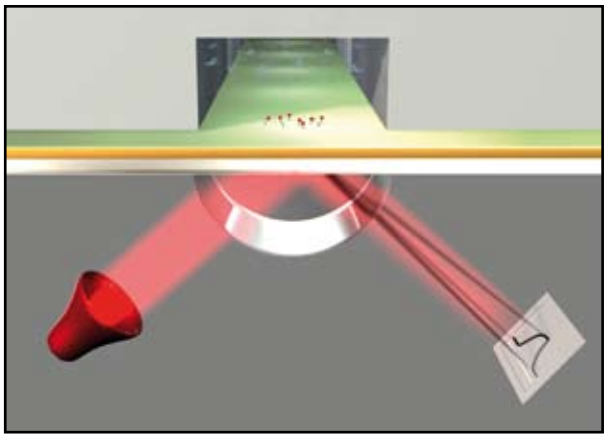

Figure 2A. As molecules are immobilized on a sensor surface, the refractive index at the interface between the surface and a solution flowing over the surface changes, altering the angle at which reduced-intensity polarized light is reflected from the supporting glass plane. The change in angle caused by association or dissociation of molecules from the sensor surface is proportional to the mass of bound material and is recorded in a sensorgram.

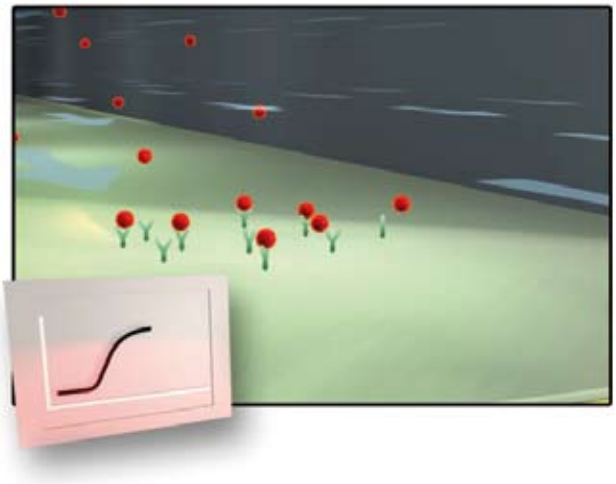

Figure 2B. When sample is passed over the sensor surface, the sensorgram shows an increasing response as molecules associate. The response remains constant if the interaction reaches equilibrium.

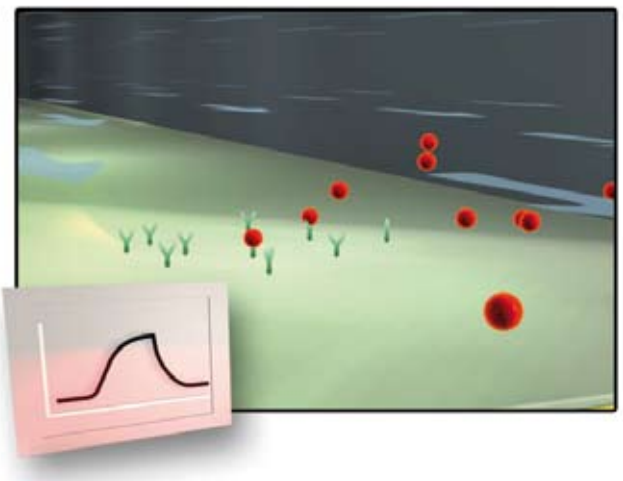

Figure 2C. When sample is replaced by buffer, the response decreases as the interaction partners dissociate. Complete interaction profiles are thus generated in real time. From these profiles, kinetics, affinity, specificity and sample concentration may be determined.

Grating-coupled SPR

GC-SPR is used to address extensive arrays spotted with hundreds of immobilized proteins, delivering simultaneous profiles on 400 interactions on one sensor surface. The sensor surface for this large-scale array (Figure 3) is prepared using a commercial spotter before insertion into the system. Incident polarized light from above strikes the entire functional face of the sensor surface, enabling simultaneous measurement of interactions on all spots and eliminating errors that could arise from sequential readings. As light is reflected from the sensor surface, evaluation software resolves the data from the individual spots into hundreds of interaction profiles. The small coupling angle of the incident light is conducive to multiple imaging and is thus well suited to screening applications.

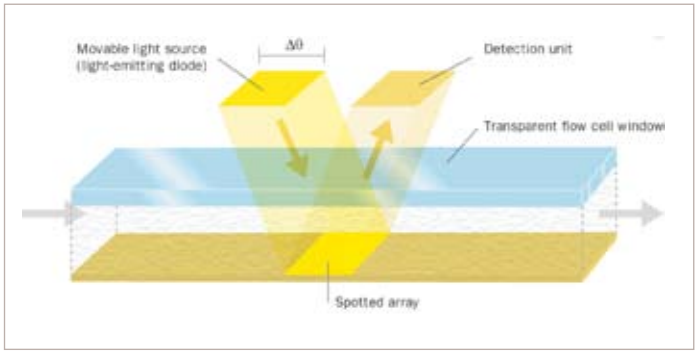

Figure 3. GC-SPR. Sample is injected through a single broad channel and interacts simultaneously with all spots on the array.

The availability of alternative label-free detection configurations enables systems to be developed to fulfill the performance requirements for a wide range of applications.

Controlling liquid flow; bringing the interactants together

Interactions occur on a sensor surface. Interacting partners in solution are delivered to the surface via a system of flow channels, formed when a microfluidic cartridge is brought onto contact with the sensor surface (Figure 4.)

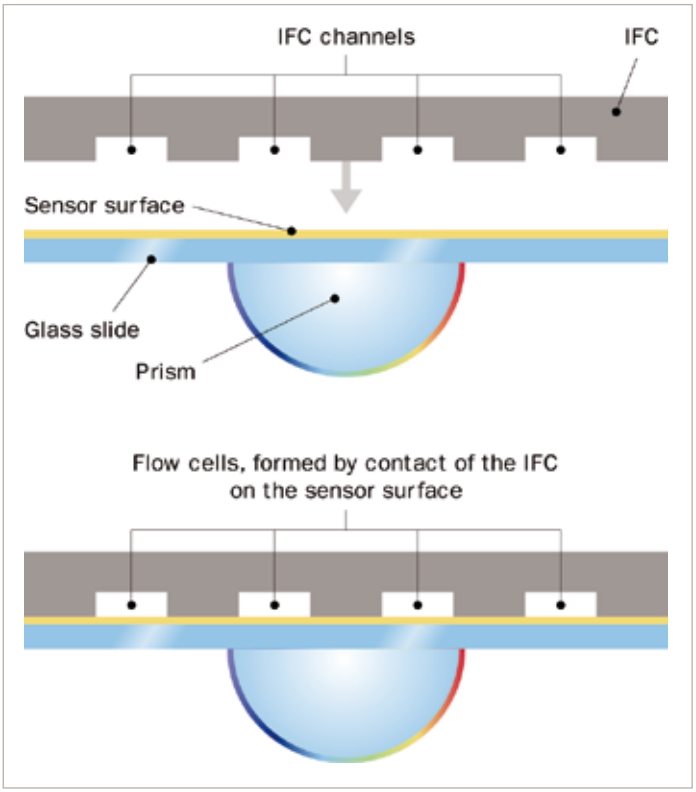

Figure 4A. Flow cells are formed in prism-based SPR detection by pressing an integrated microfluidic cartridge (IFC) against a sensor surface - the site of interaction on which one partner is immobilized and over which the other passes during sample injection.

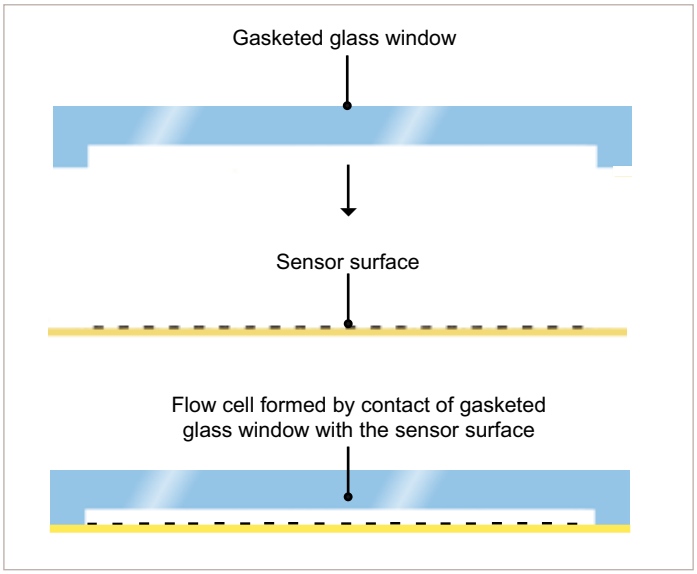

Figure 4B. Flow cells are formed in GC-SPR by hermetically sealing a gasketed window with an inlet and an outlet valve over the sensor surface.

Controlling liquid flow: flow cell design

Flow cell designs are developed to meet the requirements of the analysis.

Serial analysis: Flow cells in series (PB-SPR)

In this configuration (Figure 5), sample is injected through flow cells, which can be opened and closed by a system of valves. Interactions may be analyzed in up to four flow cells, according to the system design. Selected flow cells may be used as on-line reference cells, allowing blank-subtracted data to be presented directly, during analysis. Software-controlled valves at each outlet and at the entry to each flow cell enable the user to select a combination of active flow cells during the experiment.

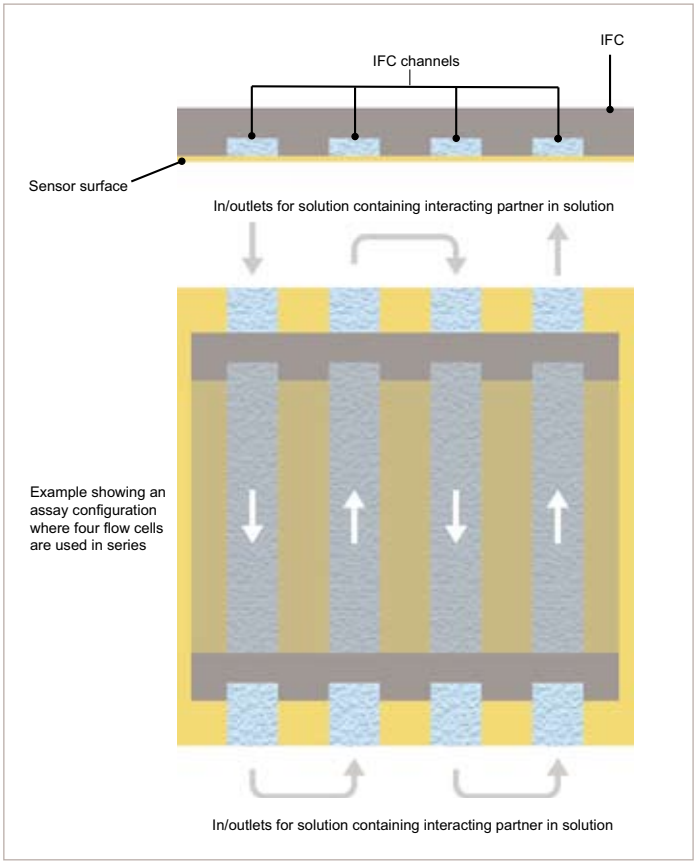

Figure 5. Flow cells in series in which selected cells may be used for on-line referencing, allowing direct background subtraction for accurate kinetic analyses

Concentration measurement: Independent flow cells (PB-SPR)

Here, sample is injected separately through independent, unconnected flow cells (Figure 6). The simpler design of this system is used in instruments dedicated to concentration measurements in which on-line referencing is less critical.

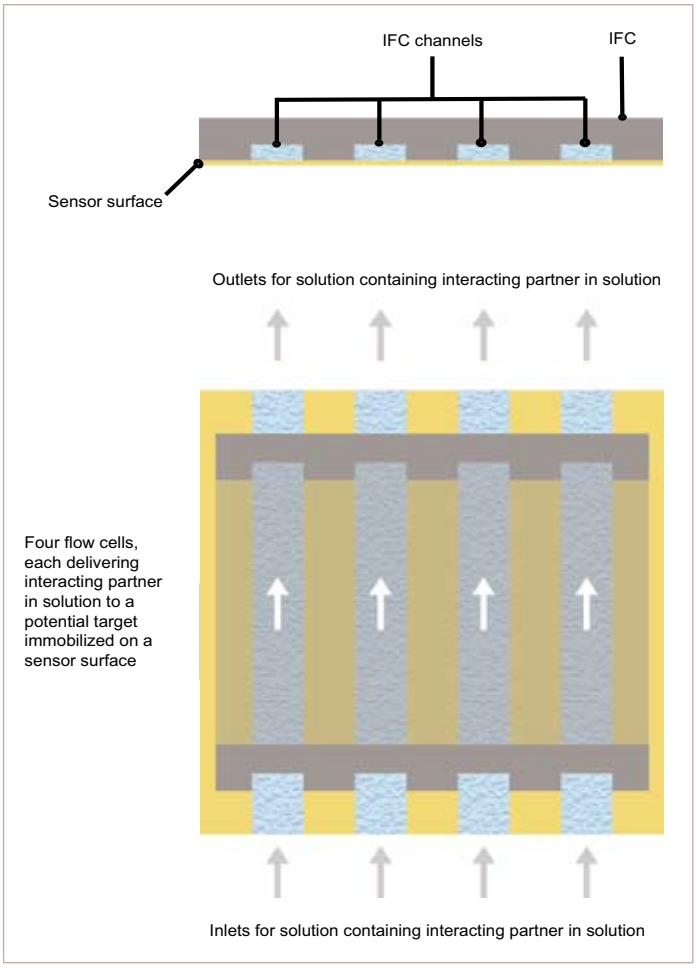

Figure 6. Independent flow cells for concentration analyses.

Simultaneous analysis: Multi-spot flow cells using hydrodynamic addressing (PB-SPR)

Hydrodynamic addressing enables multiple interactants to be immobilized on detection spots in a single flow cell, allowing simultaneous analysis of interactions (Figure 7). As there is no lag time between interactions, highly accurate reference subtraction allows the measurement of very rapid kinetics. Further, by immobilizing several interactants in one flow cell, binding properties may be directly compared under optimal experimental conditions.

By adjusting the relative flow at the two inlets (one for the immobilized partner and the other for buffer), liquid can be directed different addressable detection spots. The flow cell design allows rapid and efficient switching of flow between buffer and sample solution and the transverse arrangement of the detection spots ensures that access of sample to all spots is simultaneous. Although the detection spots are addressed separately during immobilization, the injected sample flows over all spots simultaneously.

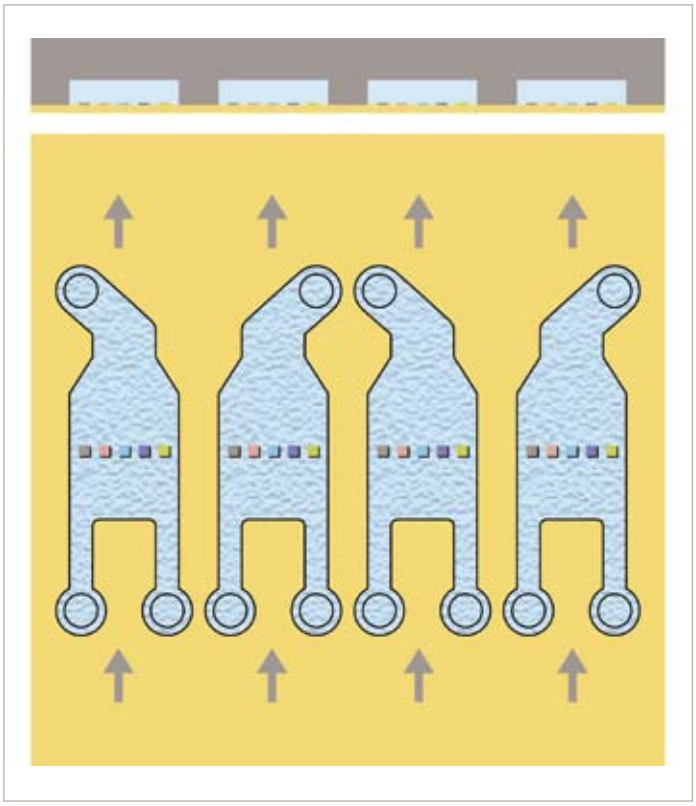

Figure 7a. Multiple spots in each flow cell

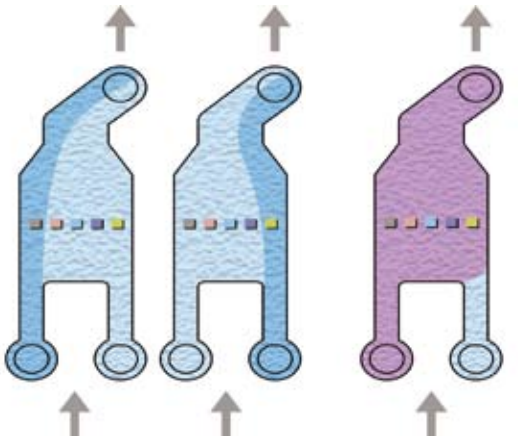

Figure 7b. i) Immobilization of interactant to spot 1 ii) Immobilization of a different interactant to spot 5 iii) Sample flows over all spots simultaneously.

Simultaneous analysis: Single pass, multi-spot flow cells (GC-SPR)

The flow cell for GC-SPR-based systems contains a single broad channel through which sample is injected, interacting simultaneously with all spots on an array (Figure 8). A gasketed window with an inlet and an outlet valve is then positioned and hermetically sealed over the sensor surface to form the flow cell.

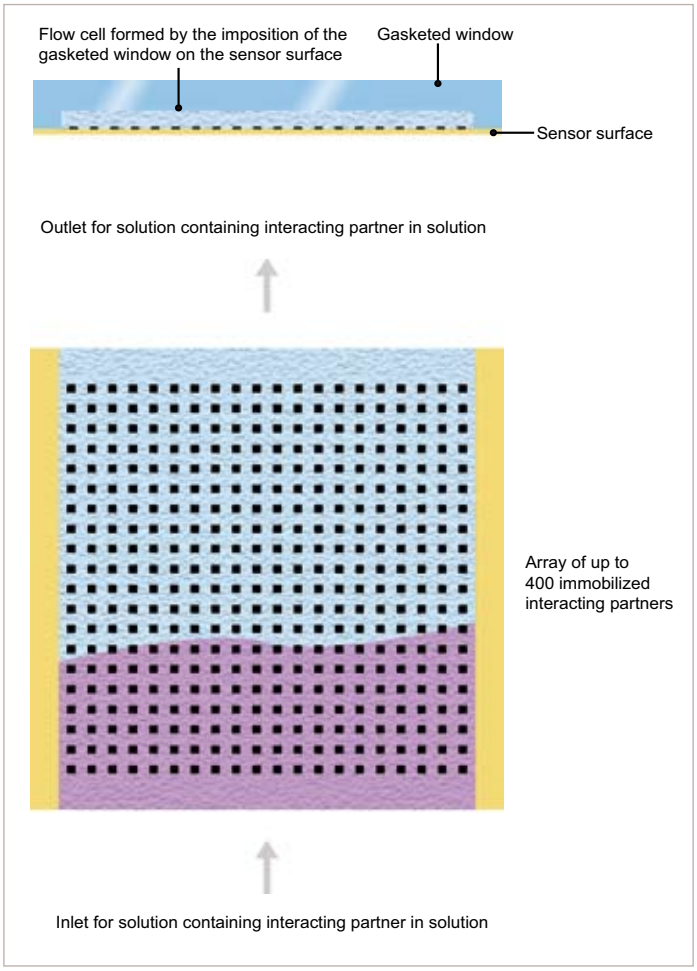

Figure 8. Single pass, multi-spot flow cells for simultaneous profiling of up to 400 interactions.

Sensor surfaces

To study an interaction, one of the binding partners is immobilized on the gold surface of a sensor chip (Figure 9). Immobilization occurs by direct coupling to the surface or via a capture molecule. A range of chips ensures that the most suitable sensor surface can be chosen according to the nature of the molecule to be coupled and the requirements of the analysis (Figure 10). Interactions are monitored by injecting samples in solution over the prepared sensor surface.

Immobilization on sensor chips for use in prism-based systems is carried out with the chip inserted in the instrument. Between injections, the surface may be regenerated by selective dissociation of the interaction partners. Regeneration solutions should ensure complete dissociation, without affecting the binding characteristics of the immobilized partner. Sensor chips for GC-SPR-based systems are prepared externally using a commercial spotter before insertion into the system.

Figure 9. Sensor chips for use in a wide range of applications.

Direct immobilization

The most commonly used surface is a matrix comprised of carboxymethyl groups either directly attached to the gold surface or covalently attached via dextran linkers of variable length (Figure 10A). The carboxymethyl groups are open to covalent amine, thiol or aldehyde coupling. Although interaction analysis assays are ostensibly solid phase, flexible dextran linkers provide a scaffold on which interactions proceed under conditions that closely mimic a fluid environment, allowing the immobilized partner considerable freedom of movement.

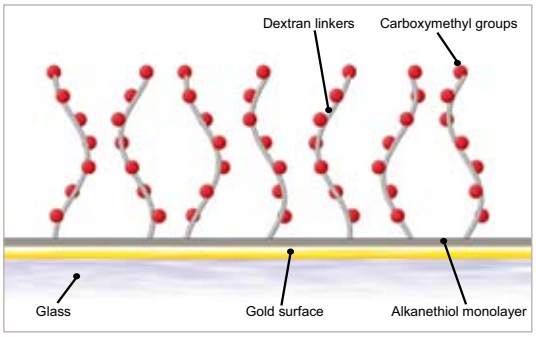

Figure 10A. An example of a sensor surface designed for direct, covalent immobilization of interacting partners.

Capture

A capture system may be used when it is desirable to immobilize binding partners in a specific orientation, where the immobilization process has been shown to partly compromise activity or where the binding partner is not biochemically compatible with a carboxymethylated surface (Figure 10B). Sensor chips are available for immobilizing biotinylated peptides, proteins, nucleic acids or carbohydrates or for histidine-tagged molecules via Ni^{2+}/NTA chelation. Note that capture systems are also possible to construct using carboxymethylated surfaces by firstly immobilizing a specific capture reagent such as a species-specific anti-IgG antibody by covalent means.

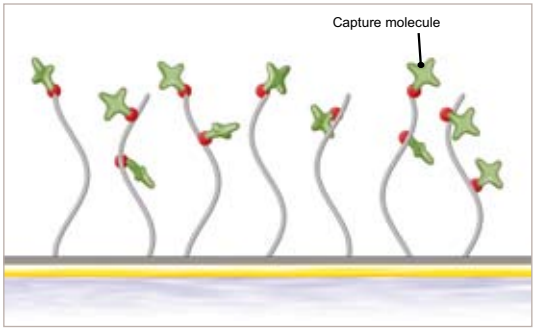

Figure 10B. An example of a sensor surface designed for immobilization of interacting partners via a capture molecule.

Immobilization of liposomes or model membranes

Hydrophobic surfaces are available for direct attachment of lipid membrane vesicles such as liposomes, for immobilization via incorporation into a lipid bilayer or for working with model membrane systems (Figure 10C).

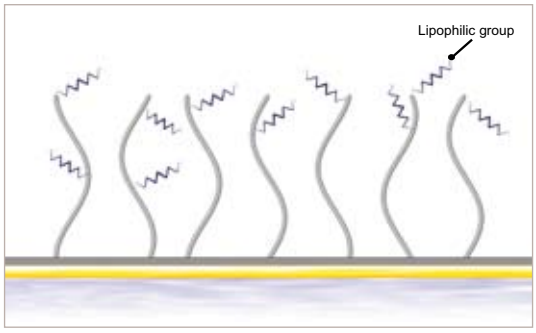

Figure 10C. An example of a sensor surface designed for immobilization of hydrophobic interacting partners such as liposomes or membranes.

Immobilization on sensor chips for use with systems using GC-SPR detection

Chips are available for direct adsorbtion of proteins, thiolated peptides or SH-modified nucleic acids, for capture of biotinylated proteins, peptides, nucleic acids and other biotinylated molecules and for immobilization of antispecies immunoglobulin, enabling the capture of mouse or human antibodies.

Further reading

Many excellent technology-focused reviews can be found in the literature. A regularly updated listing of published work utilizing Biacore systems and more information about the technology and its applications are available at www.biacore.com.

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