

# Surface plasmon resonance: towards an understanding of the mechanisms of biological molecular recognition

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With the introduction of new instruments and improved sensor chip chemistries, surface plasmon resonance (SPR) is finding new applications for molecular interaction studies. Easy access to high-quality kinetic and thermodynamic data for macromolecular binding events is providing insights into the fundamental mechanisms of molecular recognition. Progress is being made to allow larger-scale interaction studies. In addition, combining SPR with other analytical methods is enabling SPR-based analysis of interaction proteomics.

## Addresses

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## Abbreviations

CPWR couple plasmon-waveguide resonance  
EGF epidermal growth factor  
IgE immunoglobulin E  
MHC major histocompatibility complex  
SPR surface plasmon resonance

## Introduction

The biological functions of most macromolecules depend on their ability to interact with other molecules. In the post-genomic era, one of the greatest challenges facing the chemical biology community is a complete description of the interaction proteome. This information will provide insights into the mechanisms of biological processes and provide opportunities for controlling these processes by interrupting key molecular interactions.

Surface plasmon resonance (SPR) is a method for characterizing macromolecular interactions. It is an optical technique that uses the evanescent wave phenomenon to measure changes in refractive index very close to a sensor surface. The binding between an analyte in solution and its ligand immobilized on the sensor surface results in a change in the refractive index. The interaction is monitored in real time and the amount of bound ligand and rates of association and dissociation can be measured with high precision. Although SPR is a relatively new biophysical method, with the first commercial instrument being introduced in 1990, its growth has been rapid. In the past several years, SPR has taken its place as a mature biophysical technique for the analysis of molecular recognition events. SPR biosensors have become standard instruments in biochemical and biophysical research centers. There have been yearly increases in the number of publications in which SPR data is reported in an ever-wider variety of

biological systems, and recently there has been an expansion of applications into previously rare areas.

In an age where fully sequenced genomes offer a wealth of potential opportunities for insights into the molecular basis of biological processes, our next great challenge is the development of a complete set of proteomic interaction maps. SPR can contribute to these efforts with rapid and quantitative analysis of molecular interactions.

## SPR instrumentation and biosensor chips

A number of commercial SPR biosensor instruments are available [1]. Although BIAcore systems have dominated the market since their introduction [2•], there are many more competing SPR instruments now available, including new systems introduced recently by Texas Instruments and Aviv [3,4••]. A list of manufacturers and their web addresses can be found in Table 1.

Since the introduction of their instruments, both BIAcore and Affinity Sensors have marketed sensor chips with a carboxymethylated dextran matrix and a streptavidin-derivatized surface. Although thousands of publications demonstrate the versatility and robustness of these surfaces, some systems are simply not compatible with these immobilization strategies or sensor surface chemistries. Consequently, several new commercial sensor chips have been introduced in the past few years, permitting new SPR-based applications. The new surface chemistries include a carboxymethylated surface with a reduced charge on the dextran matrix, several dextran-free 'flat' surfaces with different chemistries for immobilizing ligands, a chelated nickel surface for binding His-tagged ligands, and several hydrophobic surfaces designed to allow assembly of lipid monolayers and analysis of membrane systems [5,6].

## SPR for studying biomolecular interactions: insights into the mechanisms of molecular recognition

Real strengths of the SPR biosensor technology are its versatility and ease of use. It permits the analysis of receptor–ligand interactions with a wide range of different molecular weights, affinities and binding rates, and is compatible with a myriad of different chemical environments.

SPR is effective in studying interactions for a large range of molecular weights of analytes. Experiments with analyte masses ranging from hundreds of daltons [7] to whole-cell binding [8] have been performed. Although the effective affinity range of SPR has often been quoted to range from nanomolar to micromolar, it is possible to extend this range

substantially, from sub-picomolar to greater than millimolar affinities [9]. The difficulties with measuring very high affinity interactions are generally related to very slow dissociation rates; for example, when  $k_{\text{off}} = 10^{-6} \text{ s}^{-1}$  it takes approximately  $10^4$  seconds for 1% of bound material to dissociate. Measuring these small signal changes is experimentally challenging. High-affinity interactions can instead be characterized using long sample injection times and measuring equilibrium binding conditions over a range of concentrations [9]. Care must be taken when measuring binding interactions at very low affinities [10], when specific binding may be small compared with non-specific binding to the sensor matrix or surface and instrument noise and drift. Practical advice on improving data-collection methods, running proper controls and optimizing data analysis methods has been reviewed recently [11\*\*]. The 'double referencing' technique, a method for subtracting reference data to remove small systematic signal deviations, is particularly helpful in the analysis of weak binding interactions or low molecular weight analytes [11\*\*].

SPR interaction analysis can be performed over a wide range of chemical and environmental conditions (temperature, ionic strength, pH etc.). Analyzing binding kinetics and thermodynamics over a range of different conditions can give unique insights into binding reactions mechanisms. Andersson *et al.* [12] have described a systematic approach for exploring buffer space, both as a means for regenerating surface sensors and as a means for deriving information about the binding event. Several groups have measured binding affinities over a range of ionic strengths; these studies describe the role of electrostatics for the interaction [13,14]. The Debye–Huckel plot — a plot of  $\log(K_A)$  versus  $\log(\text{ionic strength})$  — provides information on the number of ions displaced during the binding event [15]. One of the discriminating characteristics between specific and non-specific protein–DNA binding is the difference in their ionic strength dependence [16]. The binding affinities of non-specific protein–DNA interactions are highly dependent on ionic strength, because of the dominance of the electrostatic contributions mediated by the negatively charged phosphates of the DNA backbone. Binding rates and affinities measured at different temperatures also provide information on thermodynamic parameters of the interaction. In addition to the well known van't Hoff plots (providing thermodynamic parameters  $\Delta H$ , the change in enthalpy, and  $\Delta S$ , the change in entropy), the temperature dependence of the on- and off-rates can give a direct value for the activation energies ( $\Delta H^\ddagger, \Delta S^\ddagger, \Delta G^\ddagger$ ) of these processes [17].

It has long been appreciated that protein folding events can be described by complex energy landscapes and many examples of intermediates along these folding pathways have been characterized [18]. In contrast, the energy landscapes of molecular binding events are less well characterized, although they are thought to be similar in nature [19]. The ease of measuring the thermodynamic

**Table 1**

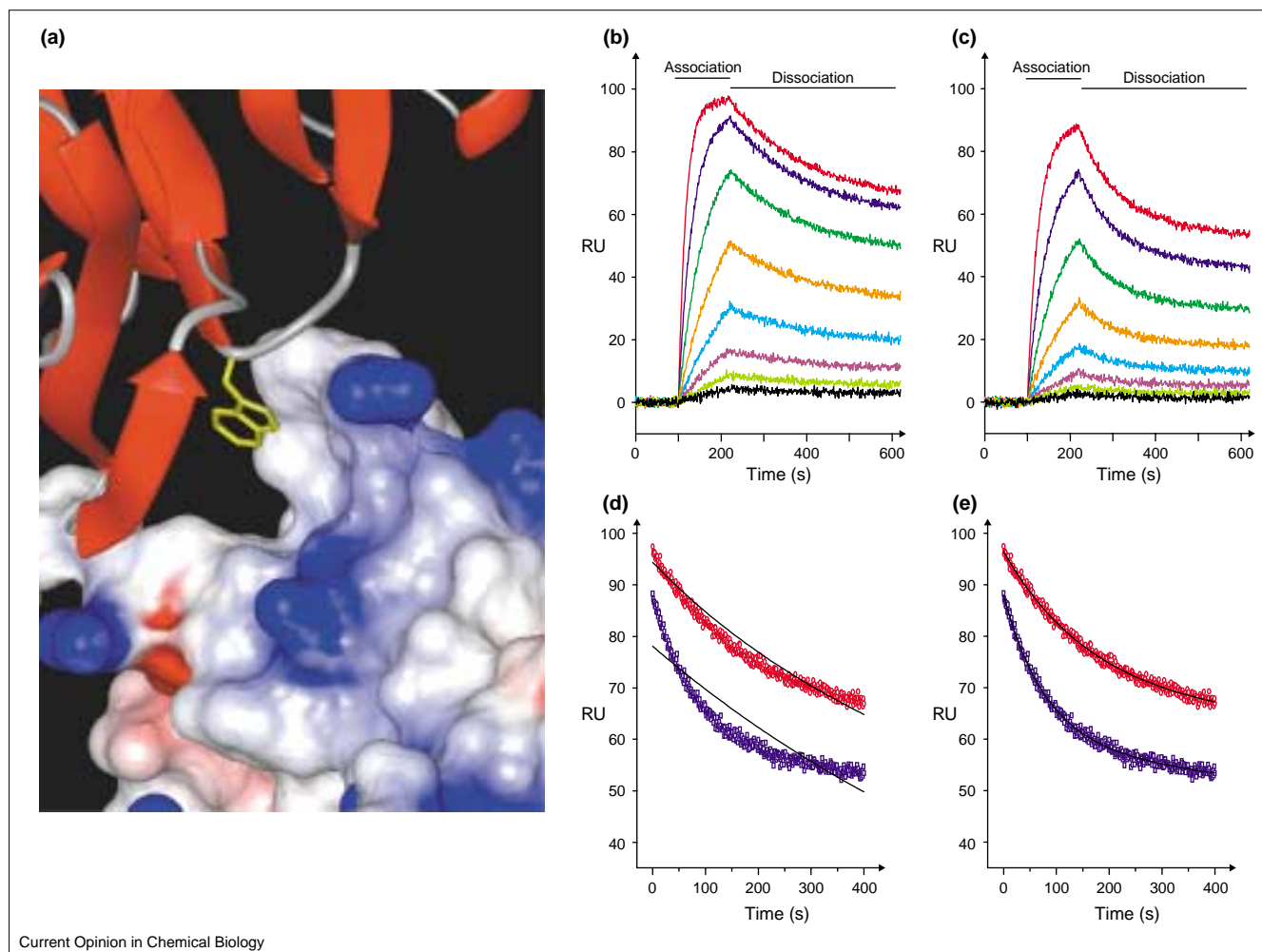
**Manufacturers of SPR instruments.**

SPR manufacturer Internet address	System
BIACore AB (Uppsala, Sweden) <a href="http://www.biacore.com">http://www.biacore.com</a>	BIACore
Affinity Sensors (Franklin, MA) <a href="http://www.affinity-sensors.com">http://www.affinity-sensors.com</a>	IASys
Nippon Laser Electronics (Hokkaido, Japan) <a href="http://www.riken.com">http://www.riken.com</a>	SPR-670
Artificial Sensing Instruments (Zurich, Switzerland) <a href="http://www.microvacuum.com/products/biosensor">http://www.microvacuum.com /products/biosensor</a>	OWLS
IBIS Technologies BV (Enschede, The Netherlands) <a href="http://www.ibis-spr.nl">http://www.ibis-spr.nl</a>	IBIS
Texas Instruments (Dallas, TX) <a href="http://www.ti.com/spreeta">http://www.ti.com/spreeta</a>	TISPR
Aviv (Lakewood, NJ) <a href="http://www.avivinst.com">http://www.avivinst.com</a>	PWR-400
BioTul AG (Munich, Germany) <a href="http://www.biotul.com">http://www.biotul.com</a>	Kinomics
Quantech Ltd (Eagan, MN) <a href="http://www.quantechltd.com">http://www.quantechltd.com</a>	FasTraQ

parameters and the energy barriers for on- and off-rates by SPR promises new insights into these processes. Using kinetic and thermodynamic information, collected over a range of temperatures and ionic strengths, Frisch *et al.* [20\*\*] have tentatively assigned a transition state for the initial interaction between barnase and barstar. Several groups have recently used SPR methods to characterize the activation energies for both association and dissociation events [21,22]. These kinds of studies will be critical in understanding the energy landscapes that control macromolecular interactions.

Analysis of kinetics and thermodynamics by SPR can be used to understand the complex mechanisms of molecular recognition events. Myszkka *et al.* [23\*] combined SPR with isothermal titration calorimetry, analytical ultracentrifuge, and structural information from X-ray crystallography to describe the interaction between CD4 and gp120. Their data suggest extensive structural rearrangements upon ligand binding, which may have implications for HIV immune evasion and viral entry mechanisms. De Crescenzo *et al.* [24] observed complex binding kinetics for the interaction between TGF- $\alpha$  and the epidermal growth factor (EGF) receptor. They considered several binding mechanisms and found the biosensor data fit best to a conformational change model. According to this model, ligand binding induces a conformational change in the receptor resulting in receptor dimerization. This mechanism is consistent with other biophysical studies of EGF receptor–ligand interactions and is thought to be important for EGF receptor-mediated cellular activation. Gunnarsson *et al.* [25] used SPR to study conformational variants of human  $\alpha 2$ -macroglobulin, identifying a

Figure 1



Insights into molecular recognition processes using SPR. (a) In the interaction between IgE and FcεRIα, Trp87 makes important energetic contributions to binding. BIAcore SPR analysis of the Cε3-4 domains from IgE binding to (b) FcεRIα wild type and (c) a Trp87Asp mutant demonstrate the effect of this mutation on interaction kinetics. Wild type (red circles) and mutant (blue squares) dissociation curves are fit (black line) to (d) a single-component exponential decay or (e) a two-component

system. The IgE–FcεRI interaction shows biphasic kinetics [26], but while the second component makes only a minor contribution to the wild-type interaction, this biphasicity is markedly exaggerated by the Trp87Asp mutation. The SPR data demonstrate the effect of this mutation on the free energy ( $\Delta G$ ) of binding, and also offer insights into the complex energy landscape of this binding event, describing the pathways used in the transition from free to bound states. RU, resonance units.

site involved in exposure of a ligand recognition site. Deviations from ideal 1:1 Langmuir models have also been observed in a large number of other systems and often this information has been used to propose mechanisms for ligand-mediated activation events [26,27] (see Figure 1). Several examples have been identified in which interaction rates are more descriptive of a given biological process than the equilibrium binding affinities. For example, Leferink *et al.* [28] have described growth factor interactions with ErbB-1 in which the dynamic rate constants correlate better with mitogenic activity than do the equilibrium constants. McDonnell *et al.* [29] identified a role for the Cε2 domain from immunoglobulin E (IgE) in allergic responses. Removal of this domain from IgE has a small effect on the overall affinity of IgE for its receptor FcεRI but has a marked effect

on the off-rate. Cε2 thus contributes to the unusually slow off-rate of IgE, which is an important factor in IgE-mediated mast cell sensitization as part of the allergic response.

The analysis of complex kinetics has been aided by the introduction of improved data-fitting software. In addition to manufacturer-supplied curve-fitting software, several excellent freeware programs are also available including CLAMP (<http://www.cores.utah.edu/interaction/clamp.htm>) [30] and SPRevolution (<http://www.bri.nrc.ca/csrg/equip.htm>) [24]. Using sensible experimental design and data collection methods [31], and applying the improved data analysis, including modelling mass transport effects [32], one can essentially eliminate experimental artefacts, which were common in early SPR studies [33,34], and allow

confidence in interpreting kinetic data into insights into biological mechanisms.

### SPR in membrane studies

Before SPR gained popularity as a technique to analyze biomolecular interactions, plasmon resonance spectroscopy was used for many years by material scientists for measurements of surface and optical properties of molecular films and interfaces [35,36]. It seems only natural, then, that SPR could be a powerful approach for studying biological membrane events. The recent introduction of sensor surfaces specifically designed to allow study of membrane interactions has resulted in a large number of new studies of membrane-associated systems by SPR. The planar nature of the sensor surface and the ability to specifically orient immobilized ligands may make SPR a better membrane surrogate than traditional solution studies. Erb *et al.* [37] have characterized one of the new BIAcore sensor chips (L1) designed for liposome absorption. Using atomic force microscopy and fluorescence microscopy, they have demonstrated that lipids form a homogeneous monolayer on the surface of the chip, suggesting a successful membrane mimic. Liposome-covered sensor surfaces have been used in an assay for lipid absorption for a panel of 27 drugs and showed a strong correlation with passive intestinal absorption [38]. Celia *et al.* [39] established a lipid monolayer using a chelated-nickel lipid and immobilized and oriented a major histocompatibility complex (MHC) molecule through a His-tag tail, and then measured binding events between the T cell receptor and the oriented MHC molecule. X-ray diffraction studies of the two-dimensional crystals of the monolayer-bound MHC molecules established that the protein had maintained the desired orientation. Several recent reports describe the characterization of membrane-integrated G-protein-coupled receptors [40–42]. Using a variation of SPR on a home-built instrument (the predecessor of the commercial system from Aviv) Salamon *et al.* [4••] used coupled plasmon-waveguide resonance (CPWR) spectroscopy to study interactions and conformational change in the rhodopsin–transducin system. The CPWR spectrum has a higher information content than the traditional SPR measurement, which typically records only a change in the resonance angle. CPWR may have some important advantages in the study of anisotropic membrane systems.

### SPR in proteomics and drug discovery

The general versatility of SPR methods, the ease of automation, and the lack of labeling requirements make it a promising tool for large-scale screening for binding events, both for small molecules in drug discovery efforts or for macromolecules in large-scale ligand fishing experiments. The improved sensitivity of new biosensor instruments routinely permits the detection of small molecular weight (<500 Da) analytes, even for low-affinity interactions ( $K_D > 1$  mM). Adamczyk *et al.* [43] used SPR as an immunoassay to monitor binding of thyroxin analogs to an immobilized monoclonal antibody. The La Jolla Pharmaceutical Company has used SPR in the clinical

development of a new drug for the treatment of the autoimmune lupus [44]. A review by Myszkla and Rich [45] discusses recent progress in SPR in drug discovery efforts. This report also describes a prototype microarray chip, a sensor surface with 64 individual immobilization sites in a single flow cell (the standard BIAcore chip has four independent flow cells). SPR-based arrays offer the opportunity to move towards larger-scale matrices of receptor–ligand interactions, the type of analysis that will be required to build complete proteome-interaction maps. A great number of different protein biochip technologies are being pursued [46•,47–49]. One promising approach is the combination of protein-chip-based technologies with mass spectrometry (MS) (see for example [50]). Several groups have integrated SPR and MS for affinity-based capture and characterization of ligands [51,52]. Nelson *et al.* [53] discuss performing matrix-assisted laser desorption/ionisation (MALDI)-MS directly on ligand-bound biosensor surfaces. A fully in-line system combining an SPR biosensor and electrospray tandem MS has been described [54•]. The SPR platform allows the detection, capture and subsequent digestion and delivery of nanomolar to femtomolar levels of ligand for MS analysis. SPR/MS is a rapid and powerful approach for identifying binding partners from complex mixtures of components. Combining this technology with larger microarrays makes it a feasible approach for large-scale ligand-fishing experiments or interaction proteomics analyses.

### Conclusions

With the introduction of a number of new SPR instruments and a series of novel sensor surfaces and chemistries, the impact of SPR biosensors on molecular interaction studies will continue to grow. The ability to form stable membrane surfaces on biosensor chips will greatly simplify binding analyses in membrane systems, making this important class of biological systems far more accessible to quantitative analysis. With improved experimental design and data analysis methods, it is now easier to obtain high-quality data for the kinetic and thermodynamic parameters of intermolecular interactions. These data promise additional insights into the mechanisms of molecular binding events, which will be important in rational drug design of inhibitors of macromolecular interactions. SPR and other protein-chip-based technologies are beginning to show promise in larger-scale interaction studies, both for small-molecule analysis and macromolecular ligand-fishing experiments. Great potential exists for interfacing SPR and MS in a broader microarray approach for characterizing proteome-wide interaction maps.

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