The first commercially viable surface plasmon resonance (SPR) biosensor was developed by Biacore AB (www.biacore.com) and became available in 1990. Over the past 13 years, we've seen explosive growth of the biosensor's use in both life science and pharmaceutical research. While the technology is now de rigueur in most research facilities, many scientists remain uniformed about SPR's breadth of applications. In addition, there has always been a sect of antagonists who doubt the validity of the biophysical results obtained from the biosensor. Here we address (with a focus on pharmaceutical applications) a number of common questions regarding SPR biosensor analysis.

What is surface plasmon resonance?
SPR is an optical phenomenon that involves the transfer of light waves (photons) into electron waves (plasmons) at a thin metal layer. Plasmon waves are sensitive to the refractive index of the medium near the surface through which they travel. SPR biosensors take advantage of this property to monitor molecular interactions without labelling either reactant. One binding partner is immobilised on the surface and the other partner is flowed across it (Figure 1). As complexes form, the accumulation of mass on the surface changes the solvent's refractive index, which can be monitored in real time by the SPR detector.

What are the benefits of SPR biosensors?
No labelling requirements and real-time analysis are two key aspects of SPR technology. The lack of labelling reduces the time required to prepare samples for analysis and removes the concern that a tag may alter the reaction. Real-time monitoring makes it possible to extract detailed information about binding events, including the association and dissociation reaction kinetics. Knowing how fast a molecular complex forms and breaks down is essential, particularly in structure/activity relationship (SAR) studies. Also, compared to other label-free interaction technologies such as titration or scanning calorimetry, SPR biosensors consume less sample and have higher throughput.
Does immobilising one reaction partner on to the biosensor surface alter its binding properties?

Of course it is always a concern that immobilising a molecule on to a surface may change its binding properties by occluding the binding site or by restricting entropic freedom. However, there are a number of immobilisation chemistries commonly available that allow the immobilised molecule’s binding site to remain accessible. And in practice, the majority of biosensor experiments are performed on a surface that contains a non-crosslinked hydrogel designed specifically to minimise steric constraints and nonspecific interactions.

How do the binding constants obtained using SPR biosensors compare with those from calorimetry or stopped-flow?

Given the expanding role of biosensors in analysing small molecule/macromolecule target interactions, we characterised a small-molecule inhibitor/enzyme interaction by SPR, isothermal calorimetry and stopped-flow fluorescence. For this system we found the kinetics, affinity and thermodynamic parameters obtained from the different methods were indistinguishable. This validates the use of biosensors as a biophysical tool in drug discovery by demonstrating that immobilising the enzyme on the surface did not affect the binding constants. In general, we have found excellent correlation between solution-based and biosensor-based reaction constants, as long as the biosensor experiments are designed properly.

How are SPR biosensors used in drug discovery?

The ability to monitor nearly any kind of molecular interaction allows SPR biosensors to be used in every aspect of pharmaceutical science, from target identification and characterisation to supporting clinical trials and production of biopharmaceutical agents. In fact, it’s hard to identify another technology that can be implemented in so many aspects of the drug discovery process.

Historically, most work involving biosensors was focused on obtaining kinetic information for macromolecular interactions. It is perhaps fortuitous that SPR biosensors became available at the same time that production of recombinant proteins began in earnest. After all, there were no convenient methods available to monitor the binding activity of macromolecules.

Today, biosensor applications have expanded well beyond target characterisation to support screening, lead optimisation, early ADME, clinical trials and production. Unfortunately, many researchers are either unaware or wary of the technology’s utility in the downstream development phases.

How are the detection limits of SPR biosensors defined?

There are three ways of thinking about detection limits. One is in terms of the minimum size of the molecule detectable in solution, the second is in terms of the minimum concentration of sample that is detectable, and the third is the ability to...
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detect very fast or very slow binding events, which translates into measuring very low- or high-affinity interactions.

**What is the smallest analyte that can be detected by an SPR biosensor?**

It is important to realise that the changes in refractive index monitored by SPR detectors are directly proportional to the amount of mass at the surface. Therefore, as the analyte in solution gets smaller, the overall signal intensity will diminish. In the past, this limited the applications of SPR biosensors to studying macromolecular interactions. However, recent advances in instrument hardware, experimental design, and data processing software now make it routine to study directly small molecules binding to macromolecular targets immobilised on the surface.

In 2002 Biacore AB released the most advanced incarnation in their family of biosensors, Biacore S51. This instrument was designed with a focus on small molecule detection. The entire system has been optimised to improve the signal-to-noise ratio and sample delivery. The result is an instrument that is capable of monitoring molecules with masses less than 90Da binding directly to macromolecular targets. As such, we see this platform being adopted for secondary screening and lead optimisation analyses.

**What is the lowest analyte concentration that can be detected by an SPR biosensor?**

The lower limit of analyte detection is dependent on the mass of the molecule that is being monitored, as well as its affinity for the immobilised detection molecule. Under standard conditions when using an immobilised antibody, a typical protein antigen could be detected at levels of <1µg/mL. This level of detection is sufficient to quantitate many native serum components and to monitor expression or purification of protein products. In fact, Biacore AB has developed the first SPR-based system (Biacore C) specifically designed for GLP/GMP applications.

Perhaps the most under-utilised application of biosensor technology is in determining the concentration of a molecule in solution. Unlike UV spectroscopy or dye-based assays that detect the total amount of protein, biosensor assays can be easily configured to monitor the active concentration of a molecule, even in complex samples such as cell supernatants or sera. It is not surprising that as familiarity with these applications grows, we are seeing biosensor technology adopted in clinical assays and to support biopharmaceutical production.

**What ranges of binding kinetics are accessible with SPR biosensors?**

Under standard operating conditions, SPR biosensors...
can measure association and dissociation kinetic constants in the range of $10^2$ to $10^8 \text{M}^{-1}\text{s}^{-1}$ and 1 to $10^{-6} \text{s}^{-1}$, respectively. These ranges span the kinetics of more than 95% of the biological systems we typically encounter. The upper limit for the association kinetics is controlled by the diffusion rate of an analyte to its binding partner immobilised on the biosensor surface. The lower limit of the dissociation rate is dependent on the baseline stability of the instrument. For example, as the stability of a complex increases, as often occurs for extremely high-affinity antibodies, we need to monitor a very small decay in the binding signal that occurs over a long period of time.

What is the range of affinity that can be measured?
The range of affinities accessible with SPR biosensors spans a billion fold, from millimolar to picomolar. One powerful aspect of biosensors is the ability to measure weak or transient interactions. Unlike pull-down assays based on plates, beads, or filter binding, biosensors can detect the amount of complex formed in the presence of free material. This avoids perturbing the reaction equilibrium and makes it possible to characterise even very weak complexes that would normally be lost during washing steps. As a result, biosensor technology can be used to characterise the interactions of early leads, which often have low affinities for a target. The lower limit of affinity depends only on the solubility of the reactant: as long as it is soluble, it can be analysed. For example, we have characterised the binding of a set of compounds to a large enzyme (100,000Da) immobilised on the biosensor surface. In this case, since the compounds were highly soluble, we were able to determine the affinities for the entire panel, even for exceptionally weak binders ($K_d = 1-10 \text{mM}$).

How are the kinetic and equilibrium binding constants used to support drug development?
Resolving the association and dissociation binding kinetics provides a detailed view of complex formation. This information is particularly useful when identifying the roles that specific functional groups, both within the target as well as within a compound, play in binding. For example, Figure 3 illustrates the difference in kinetics observed for two structurally distinct small-molecule inhibitors binding to the same enzyme. Although the affinities of the two inhibitors were essentially identical, the association and dissociation kinetics differed by as much as six-fold. These differences in the functional mechanism of binding would be lost if one studied the reaction by equilibrium-based techniques only.

Kinetic and equilibrium binding constants also provide detailed information regarding the activity of a given preparation of molecule. In fact, the US Food and Drug Administration is requesting this information as a measure of functional equivalence of biopharmaceutical agents. Guidelines regarding well-characterised biologics can be found at the Center for Biologics Evaluation and Research’s website (www.fda.gov/cber).

What is the throughput of current commercial biosensor technology?
Commonly used Biacore 2000 and 3000 platforms are capable of running 100 to 200 independent assays per day, depending on the experimental conditions. The new Biacore S51 is capable of accepting samples in one 384-well plate and has faster washing and sampling capabilities, which increases its throughput over earlier systems. This level of throughput is sufficient to support secondary screening and, while the technology is not considered high.


References

How can SPR technology be used in high-throughput screening?
One approach to implementing biosensor technology in high-throughput screening involves the application of array-based biosensor technology. Graffinity Pharmaceuticals AG (www.graffinity.com) utilises small molecule libraries micro-arrayed on SPR biosensor surfaces. Instead of screening many compounds against an immobilised target, these researchers pan one target against thousands of compounds spatially resolved on a surface. Structurally related compounds are arrayed within specific patterns on the chip so that a significant amount of SAR information is acquired from the primary screen. This technology, in terms of an instrument, is not currently commercially available, however. Instead, researchers can collaborate with Graffinity to test a target of interest against Graffinity’s immobilised compound library.

What commercial array-based SPR technology is available?
Several companies are developing large-scale array-based SPR biosensors in which the user may create custom surfaces. HTS Biosystems (www.hts-biosystems.com) and Applied Biosystems (www.appliedbiosystems.com) are collaborating to build a next-generation SPR array platform. Using grating-coupled SPR, their instrument can simultaneously examine one analyte binding with up to 400 immobilised targets within a 1cm² area (Figure 4). Initial applications will focus on antibody screening, as well as peptide and nucleic acid libraries. Downstream applications may involve antibody-based chips to monitor protein expression and cellular pathways under a variety of conditions. This SPR array platform is scheduled for release later in 2003.

How can SPR biosensors be used to characterise membrane-bound protein targets?
A majority of drugs under investigation or in production today target membrane-bound proteins. Many of these proteins, however, are problematic to study in vitro because they require a membrane to maintain activity. SPR biosensors could provide an excellent opportunity to study these classes of proteins in native lipid environments. Recent work has demonstrated that G protein-coupled receptors (GPCRs), for example, can be embedded in a lipid bilayer prepared on the biosensor surface. Current studies are focused on optimising the experimental methods to generalise the application of SPR for membrane-associated systems.

How are SPR biosensors being applied in ADME studies?
All too often, promising drug leads fail at the level of bioavailability. This is particularly unfortunate because using traditional drug discovery tools, much time, effort and expense has been invested in a lead before it reaches the ADME phase in development. In addition, current ADME tests are themselves laborious, time-consuming and expensive. A number of groups have explored the potential of using the SPR biosensor as an in vitro tool for performing early bioavailability characterisation.

In one application, the biosensor surface provides an excellent in vitro mimic of membrane environments. Lipid layers of varying composition and fluidity can be prepared on the biosensor and tested...
against panels of compounds to evaluate permeability, SPR is also being used to examine the interactions of compounds with serum proteins such as human serum albumin (HSA), α1-glycoprotein, and immunoglobulins. We recently demonstrated how the biosensor can be used to evaluate a drug’s binding site on HSA. The application of SPR in metabolism and excretion studies is more problematic. Some of the difficulties include eliminating non-specific binding events often observed at high compound concentration and maintaining cytochrome P450 activity on the biosensor surface. However, progress in this area is being made.

**What is the biggest drawback of SPR biosensors?**

There are several drawbacks to the SPR biosensor technology. First, it is important to remember that biosensors by their nature depend on the availability of active biological molecules. Unlike other analytical technologies such as mass spectrometry or UV spectrometry, which can be used to characterise the material whether it is properly folded and active or not, biosensors require that the molecule be active to obtain a signal. The minimum percent activity required depends to a large extent on the goals of the study, but obviously, the higher the activity, the better the results. Another drawback is that while the biosensor instruments themselves are relatively simple to operate, designing and implementing an assay often requires a reasonable knowledge of biochemistry.

**What commercial SPR biosensors are available?**

Fortunately for us laboratory scientists, we are working at a time when biosensor instruments, along with their supporting supplies and analysis software, are readily available. A quick search of the Internet will reveal a dozen or so SPR biosensor platforms in various states of development and production. Currently, the commercial market is dominated by Biacore AB (www.biacore.com), which alone distributes seven different versions of its SPR-based biosensor technology. Many of the platforms are engineered for specific applications and we recommend visiting its website for more details on the different units. Other commercial instruments are available from Affinity Sensors (www.affinity-sensors.com) and IBIS (www.ibis-spr.com). These systems vary in their level of automation, sensitivity, and cost and therefore address different user requirements.

**What future developments in SPR biosensors do you foresee?**

Commercial biosensor technology has improved dramatically over the past 10 years. The recent advancements in hardware now provide higher resolution of small molecule/target interactions and have opened up entirely new applications. However, none of the commercially available biosensors today allow parallel processing of more than one analyte at a time. This is not a problem with the SPR optical detection systems per se, but rather an issue of sample delivery. It turns out that to rapidly deliver a reactant to a sensor surface in uniform concentration and with minimal sample volume is no trivial matter. In attempts to increase the biosensor’s throughput, several groups are engineering improved micro-fluidic systems that may be introduced into commercial platforms within the next few years. Given the expanding role of biosensors in drug discovery, we also expect to see continued improvements in sensitivity and throughput as improved detection methods and array technologies become available. The other trend that we are seeing is the development of systems and software to support dedicated applications. This will help further expand the technology into niche areas of research and development.

**What advice do you have for someone interested in purchasing an SPR biosensor to support drug discovery?**

Buy two.

David G. Myszka obtained a PhD in Biochemistry from Ohio State University in 1991 and completed a postdoc at SmithKline Beecham in King of Prussia, Pennsylvania. He has consulted with various biosensor manufacturers to improve hardware, software and assay design. He has also developed novel biosensor data processing and analysis programs and published more than sixty articles describing the technology’s diverse applications. Currently Dr Myszka is Director of the Center for Biomolecular Interaction Analysis at the University of Utah. The Center maintains a wide variety of SPR biosensors for research and development. More information about the Center’s activities and capacity for contract services can be found at www.cores.utah.edu/interaction

Rebecca L. Rich is a Senior Research Scientist at the Center for Biomolecular Interaction Analysis at the University of Utah. She obtained a PhD in Physical Chemistry from Iowa State University in 1993, after which she completed a postdoc at the Texas Medical Center in Houston, Texas. Together with Dr Myszka, she is developing new biosensor applications for all phases of drug discovery.