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Lead optimization

Why you should be using more SPR biosensor technology

Rebecca L. Rich, David G. Myszka*

Center for Biomolecular Interaction Analysis, School of Medicine Rm., 4A417, University of Utah, 50 N. Medical Drive, Salt Lake City, UT 84132, USA

There are three ways of looking at surface plasmon resonance biosensor technology: bewilderment (I can not believe this sort of technology actually exists), love (without this technology I would cry), or hate (this technology does not work). Whether you love them or hate them, or just have never heard of them, it is time to take a fresh look at how SPR biosensors can help you get from point A to point B in your drug discovery efforts.

Surface plasmon resonance (SPR) is an optoelectronic phenomenon through which real-time data of binding of ligands to their receptors can be recorded. Both kinetic data, the on- and the off-rate, are determined and from these the equilibrium dissociation constant can be calculated. Lead optimization involves improvement of the binding affinity of a potential drug to its receptor. Such improvements obviously require a detailed comparison of a series of potential candidates. Moreover, SPR has the potential to monitor how chemical modification influences binding to and dissociation from the target separately. Little material is required and no labeling must be introduced. The technique has now matured and measurements can be recorded in automated fashion on larger selections of molecules. David Myszka is the director of the Center of Biomolecular Interaction Analysis and is currently involved in developing applications of the technique for drug development. He has written several research papers and reviews in the field.

Introduction

Close your eyes. Now imagine there was a technology that allowed you to measure the interaction of nearly any molecule in real time without labeling. Sounds too good to be true? Ok, open your eyes. You might be surprised to learn that this technology has been commercially available for 14 years in the form of surface plasmon resonance (SPR)-based biosensors. Currently, these instruments are being used in nearly every aspect of the drug discovery process, from target characterization, compound screening and lead optimization to supporting clinical trials, regulatory approval and biopharmaceutical manufacturing ([1,2]; also see Related articles and Links). So if you are not using SPR technology in all these ways, why not?

What are you afraid of?

Are you afraid that the surface might affect the binding interaction? Well, we have had it up to here with this argument. ELISA, RIA, and filter-binding assays all rely on a surface, yet they have been used for years without the constant controversy biosensors have faced. Sure, if a biosensor experiment is set up wrong, the surface can affect a binding interaction. In fact, early on there were a lot of poorly performed biosensor experiments and some of the legacy data are surely suspect. But improvements in experimental design, data analysis, and user experience mean that in the right hands, the binding constants obtained from the biosensor, match solution-based measurements [3–5]. Compared to other methods like calorimetry, the biosensor requires much less material, and provides dynamic information about complex formations, and measures a much wider range of affinities. If you are dying to have thermodynamic data, you can measure binding kinetics at different temperatures automatically using the biosensor [4,5]. So, we think it is

*Corresponding author: (D.G. Myszka) dmyszka@cores.utah.edu,
URL: http://www.cores.utah.edu/interaction

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Glossary

Analyte: the binding partner in solution in an SPR experiment.
Ligand: the binding partner immobilized on the surface in an SPR experiment.
Sensorgram: biosensor data output recorded as a plot of binding response versus time.
Surface plasmon resonance: the electromagnetic wave phenomenon used to monitor changes in refractive index at metal surfaces.

time to look past the surface so that you can appreciate all the benefits this technology has to offer.

A second common complaint is that biosensor instruments cost too much. Although it is true some models can cost more than a Ferrari, there is also truth in the adage that you get what you pay for. When you talk about buying a Ferrari, you do not complain about the price because you know you are getting a high-performance piece of equipment that is designed for a certain application: to go fast. The same can be said for biosensor technology: you are buying a well-engineered machine that is designed to speed up your drug discovery effort. High-end instruments ($250,000–$500,000) are equipped with auto-samplers to increase sample throughput and data quality. On cruise control, the instrument can run unattended overnight, improving efficiency and allowing you to do more during the day. There are dedicated surface chemistries to help assay design and specialized software to assist in data analysis. When amortized over the operational life expectancy (>9 years and still counting for some of the instruments we have been using), the cost of a high-end platform amounts to ~$75/day. If the biosensor technology could decrease the time to market for your run-of-the-mill billion-dollar-per-year drug by one day, that day alone would increase the profit margin by $2.7 million, paying off the technology investment ten times over in a single day. So, it is no wonder that the most profitable pharmaceutical and biotechnology companies have the most biosensors.

And like a Ferrari, to get the most out of a biosensor requires a dedicated, well-trained, and knowledgeable operator. Who buys a Ferrari without having a driver's license? If you are going to invest in one of these instruments, you would certainly want to devote the time and effort to get someone who knows what they are doing behind the wheel. Whether you are Mario Andretti, a team leader, or a biosensor rookie, the purpose of this review is to make sure that you are taking full advantage of current biosensor technology and preview the models that will be coming to a showroom near you.

How they work

In a typical biosensor experiment, one binding partner is immobilized on a sensor surface and the other binding partner is passed over this surface in solution (Fig. 1a; see Glossary). Almost any molecule, from proteins and DNA to lipids and oligosaccharides (for those of you on a low-carb diet), can be immobilized on the sensor chip surface and monitored for binding to soluble analytes ranging in size from low-molecular-mass drugs (e.g. salicylic acid, 138 Da) to large, multi-component viral particles [6].

Currently available optical biosensors use either prism-coupled or grating-coupled SPR to measure a binding response (Fig. 1b,c). The beauty is you really do not need to understand quantum mechanics to use the technology. If anyone asks, just tell them ''an SPR detector monitors changes in refractive index that occur at the sensor surface as molecular complexes form or break during a reaction''. These changes are monitored over time and converted into a sensorgram (Fig. 1d), from which the kinetics and affinity of the interaction can be determined. In addition to measuring binding constants of purified materials and complex mixtures, biosensors can identify binding partners, map epitopes, and measure the active concentration of analyte in crude samples. In fact, excluding the Swiss Army® knife, no technology is as versatile as a biosensor.

Biacore AB (http://www.biacore.com/) released the first commercial biosensor in 1990 and they continue to be the major manufacturer of this technology. Currently, they offer several biosensor platforms that are used throughout the drug discovery and development process. In this review we highlight three classes of instruments from Biacore AB, a new sensor technology co-developed by Applied Biosystems and HTS Biosystems, and discuss the emergence of higher-throughput systems.

Five key technologies

Biacore® 2000 and 3000 platforms

Biacore 2000 and 3000 instruments have been the workhorses of life science research. Although these instruments were initially designed to characterize protein interactions, they have been applied to study membrane fractions, phage, whole cells and even small molecules [6]. Worldwide, they are the most commonly used SPR biosensors. The 2000 and 3000 instruments have four flow cells arranged in series that can be monitored simultaneously (Fig. 2a). This huge advance over the earliest Biacore platforms makes it possible to collect data from three reaction surfaces and one reference surface simultaneously. Referencing is a fundamental requirement in SPR technology because it corrects for bulk refractive index changes and nonspecific binding.

In most Biacore experiments, the ligands are not immobilized directly onto a flat surface, but rather are tethered to a carboxylated dextran matrix that coats the chip surface (Fig. 1a). The matrix provides a solution-like environment to study molecular interactions. Besides providing a point for

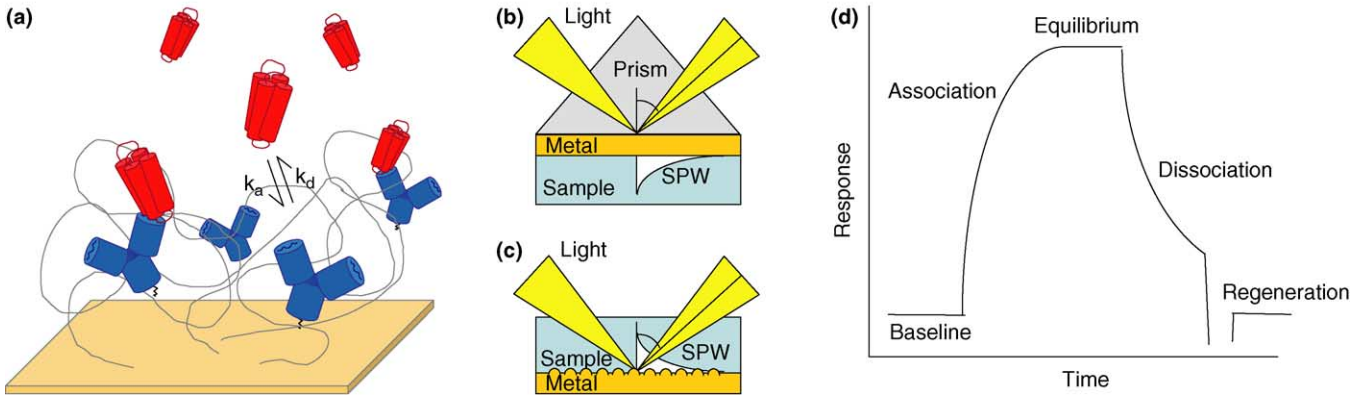

Figure 1. Key components of SPR biosensor technology. (a) Assay design of a typical biosensor experiment. A ligand (in this example, an antibody) is immobilized on the sensor chip surface and an analyte (antigen) is injected over the surface to measure the binding interaction. Schematic of the SPR detector used in (b) Biacore instruments and (c) the 8500 Affinity Chip Analyzer. (d) Biosensor data output: the initial, flat response signal indicates the instrument is stable; the response increases as analyte flows over, and binds to the surface; the response decreases as analyte dissociates from the surface during the buffer wash; and an injection of a regeneration solution, which completely disrupts residual analyte/ligand interactions, returns the response back to baseline.

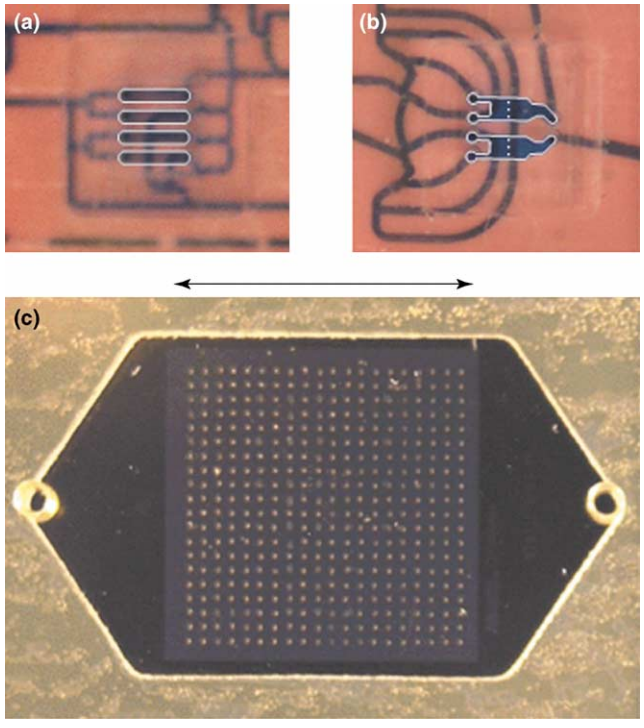

Figure 2. Images of Biacore and 8500 Affinity Chip Analyzer biosensor flow cell configurations. In this illustration, your eye would effectively be positioned as the detector to monitor the light intensity during the reaction. For perspective on the size of all three flow systems, the length of the double-headed arrow corresponds to 1 cm. (a) A black dye was used to better visualize the four flow cells arranged in series in Biacore® 2000. In an experiment, ligands are immobilized in three of the flow cells and the fourth serves as the reference. (b) Two *y*-flow cells side-by-side in Biacore® S51. In an experiment, ligands are immobilized on the two outer spots of a flow cell and the center spot serves as the reference. The microfluidic channels that bring samples to the flow cells are shown in the background in both panels (a) and (b). (c) In the 8500 Affinity Chip Analyzer™, ligands are spotted across the surface of the single, large-format flow cell (a 20 × 20 matrix is shown here). Analyte enters through the portal on the left and exits at the portal on the right.

chemical attachment, the carboxyl groups themselves act like a magnet for proteins, concentrating them at the surface and speeding up the immobilization process. Without this pre-concentration effect, immobilizations would need to be done at much higher concentrations (>1 mg/ml) to drive the chemistry. Thus, a large part of Biacore's success stems from their unique chip surfaces. It is no wonder that the person who developed the dextran matrix at Biacore AB has a reserved parking spot near the front of the building.

The 3000 is a newer line of instruments than the 2000 series; the main difference between the two being the dynamic range of the detector. The 3000 can measure interactions in higher refractive index media. This is important when you have high-density surfaces or highly refractive buffers (e.g. containing >8% dimethylsulfoxide or >10% glycerol). The 3000 is also designed to accommodate a new surface preparation unit that allows users to recover material that is bound to the chip and directly deposit it on a MALDI (matrix-assisted laser desorption/ionization) plate for mass spectrometry [7,8].

In general, the 2000 and 3000 platforms are very open systems. Using software wizards you can create elaborate user-defined assays. For example, it is possible to switch flow paths change temperature, injection sequences, and flow rate in an automated run. Fig. 3a illustrates how the instrument can be used in a multi-step analysis of human growth hormone and its receptor: hormone was first captured by immobilized receptor and then the kinetics of soluble receptor binding to the complex on the surface were measured [9]. Besides their use as biophysical tools, these instruments have found a place in drug discovery for characterizing the activity of proteins destined for screening assays, structural analysis, or pre-clinical studies.

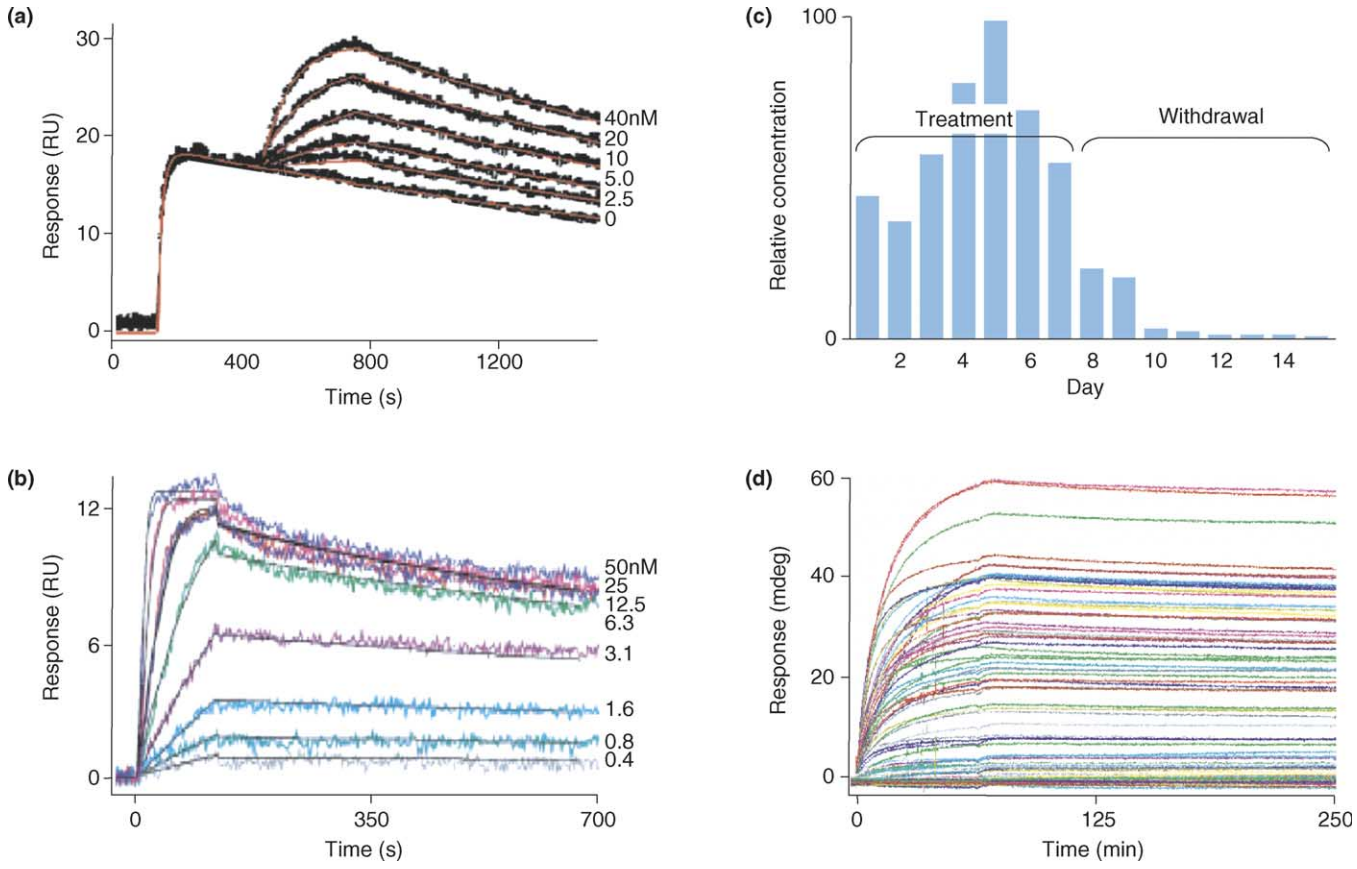

Figure 3. Example data sets obtained from the different SPR technologies. (a) Kinetic analysis of human growth hormone receptor homodimerization using Biacore 2000. The red lines depict the fit of the data to a simple interaction model to yield kinetic and affinity parameters. Adapted from [9] with permission (Copyright 2003, National Academy of Sciences, USA). (b) Kinetic analysis of a small molecule/protein interaction using Biacore S51. The black lines depict the fit of the data to a simple interaction model to yield kinetic and affinity parameters. Adapted from [10] with permission (Copyright 2004, John Wiley and Sons Limited). (c) Relative ractopamine concentration in cattle urine monitored over 15 days using Biacore Q. Figure constructed from data presented in Table 2 of [11] with permission (Copyright 2003, American Chemical Society). (d) Antigen binding to 100 different monoclonal antibodies using the 8500 Affinity Chip Analyzer (D.G. Myszka, unpublished).

Biacore® S51

Biacore S51 instruments were engineered from the initial design stages to characterize small molecule interactions in a routine way. In comparison to the 2000 and 3000 platforms, nearly everything about the S51 has been further optimized. The most dramatic change is the use of a hydrodynamic-addressing flow cell (Fig. 2b). This flow cell has two inlets and one outlet. By carefully controlling the flow rate through the two inlets, during the immobilization phase it is possible to deposit molecules within different regions of the flow cell while leaving the center unmodified as a reference spot. Having an internal reference improves system performance and data quality. The system also has improved robotics and sample introduction, which increase throughput and make it easier to deal with compounds having limited solubility, requires only 2–5 μg ligand to create a reaction surface, and consumes analyte volumes of 50–100 μL. With these advancements we can now efficiently examine molecules smaller than 100 Da [19].

Fig. 3b demonstrates the power of Biacore S51 to monitor a small-molecule inhibitor (629 Da) binding to HIV-1 protease to obtain rates of association and dissociation, as well as affinity information [10]. Binding kinetics provide a new and detailed view into the structure-activity relationship for these target–inhibitor interactions. Traditionally, lead compounds have been optimized based on their affinity alone. Kinetic analyses can reveal precisely how changes in structure affect the association and dissociation rate of a potential agonist or antagonist.

With a throughput of one 384-well plate per day, Biacore S51 is an ideal platform to support secondary screening. Not only can it provide evidence for direct interactions between a compound and a target, but it can also help to determine if a compound binds nonspecifically or nonstoichiometrically to a target, which is essential in validating hits from a primary screen. Currently, Biacore S51 is the most expensive biosensor on the market and, coincidently, it boasts a candy-apple red paint job.

Biacore® C and Q, and GxP software

Biacore C and Biacore Q have a chassis similar to that of the Biacore 2000 and 3000 series of instruments, but these units have dedicated methods, reagents, and software for concentration analyses. Biacore C is the first SPR-based system to be specifically designed for GLP/GMP/GCP (good laboratory/manufacturing/clinical practice) applications. Compliant systems must meet certain criteria established by the United States Food and Drug Administration (FDA)'s Title 21 Code of Federal Regulations Part 11, which means the software has electronic auditing, signatures, and lockdown (all of which is geared to reduce our dependence on paper records and improve data security in the electronic age).

Biacore Q is dedicated to the analysis of food products [11–14]. There are several pre-established reagent kits to test for a variety of veterinary drug residues, antibiotics, growth promoters, and vitamins. The tests are easy to perform and data analysis is fully automated. As an example, Fig. 3c shows how the Q was used to track the concentration of a feed additive, ractopamine, in cattle urine over several days [11].

Biacore C is engineered for the biopharmaceutical industry. The C is being used to determine concentrations of antibodies in titers and protein-based drug levels in body fluids, to establish expression levels of recombinant macromolecules in culture, and to maintain quality control [15] (Biacore Application Note 38 and Biacore Technology Note 19, http://www.biacore.com/). For example, scientists at Micromet AG recently developed a Biacore C-based assay to determine the concentration of monoclonal antibodies under production for the treatment of prostate cancer [Biacore Application Note 38, http://www.biacore.com/].

Importantly, biosensors can determine the active analyte fraction, not just its total concentration. Another advantage is that the sensor can pick up even weakly associating antibodies, which might be responsible for immunogenic responses in patients [16]. In many cases, these antibodies would be missed in a traditional ELISA because the complexes would dissociate during the washing steps.

GxP software for the Biacore 3000 platform is also now available, so you can turn your biophysical biosensor into a lean-mean-FDA-approved data-collection machine. Coupling the compliant data handling and storage package with the 3000's versatility and kinetic capabilities has increased this platform's utility in pre-clinical and clinical applications [17]. We think it is great to see instrument manufacturers responding to requests from end users for dedicated platforms, as well as requirements of regulatory agencies for compliant systems.

8500 Affinity Chip Analyzer™

In an effort to increase the throughput of biosensor-based kinetic analyses, Applied Biosystems (http://www.appliedbiosystems.com/) and HTS Biosystems (http://www.htsbiosystems.com/) recently co-developed the 8500 Affinity Chip Analyzer. This platform relies on grating-coupled SPR (Fig. 1c) and employs a single large flow cell (Fig. 2c) in which up to 400 ligands can be spotted and analyzed at one time. This ''one on many'' format allows one analyte to be flowed across all the spots simultaneously to monitor complex formation in real time.

The 8500 Affinity Chip Analyzer is particularly well suited to examine antibody–antigen interactions (see Applied Biosystems Application Notes about antibody characterization at http://www.appliedbiosystems.com/). A panel of purified antibodies can be spotted directly onto a chip or antibodies can be captured out of crude hybridoma supernatants onto a chip over-coated with something like Protein A. Fig. 3d shows an example of testing one antigen binding to 100 different monoclonal antibodies. It is capable of detecting analytes having molecular masses down to around 5000 Da.

Several groups are working on creating high-content biomarker chips with antibodies against cytokines, growth factors, chemokines, and hormones for diagnostic screening of serum samples. Similarly, this platform is contributing to the high-resolution, high-throughput screening and kinetic characterization of DNA fragments and peptides (see Applied Biosystems Application Note about peptide epitope mapping at http://www.appliedbiosystems.com/). Oligonucleotide arrays can be used to determine the residues important for transcription factor interactions and peptide arrays can be used to map linear epitopes or to support functional analysis of protein interactions.

Preparing antibody, peptide, and DNA chips have proven to be relatively straightforward because these ligands retain native structure throughout the drying and reconstitution steps involved in spotting. Creating chips of more labile enzymes and receptors, however, might require more elaborate patterning methods. The 8500 Affinity Chip Analyzer certainly opens up new possibilities for biosensor analysis.

Emerging technologies

When people ask ''what is missing in the biosensor technology garage?'' we tell them that to go really fast, we need to start implementing parallel sample processing. All the instruments we have highlighted up to this point test one analyte at a time. It turns out that the hardest part of parallel processing is not the detectors, but the fluidics. Getting separate samples to the detector in a rapid manner and at constant concentration is no easy task. Here is a real-world problem that the microfluidics community could help solve.

One approach to parallel processing systems is to not use fluidics at all, but to use open cuvettes instead. SRU Biosystems' (http://www.srubiosystems.com/) BIND™ biosensor uses special 96- or 384-well plates with a colorimetric resonant grating on the bottom. The system employs a guided-mode resonant filter to monitor refractive index changes at

Table 1. Comparison summary table

Name of specific type of technology	Biacore 2000	Biacore S51	Biacore C	8500 Affinity	BIND biosensor	Biacore array platform
	Biacore 3000		Biacore Q	Chip Analyzer		
Names of associated companies and company websites	Biacore AB http://www.biacore.com	Biacore AB http://www.biacore.com	Biacore AB http://www.biacore.com	Applied Biosystems http://www.appliedbiosystems.com	SRU Biosystems http://www.srubiosystems.com	Biacore AB http://www.biacore.com
Pros	Versatile	High sensitivity (<100 Da)	GxP compliant	High ligand throughput	Parallel processing	High throughput
	Many chip types and immobilization chemistries	Reaction and reference spots in parallel	Defined protocols, software, and kits available	Reaction and reference spots in parallel	High throughput	High sensitivity
	3000 has GxP-complaint software and analyte recovery hardware	Accommodates 384-well plates		Sample recirculation for long contact times	Plates fit easily in robotics of drug discovery pipeline	
Cons	Low throughput	Not very open system	Low throughput	Sensitivity (>5000 Da)	Not available yet	Not available yet
	Reaction and reference flow cells in series			External ligand spotting		
References	[4–9,17,18]	[5,10,19]	[11–15]	See manufacturer's website	[20,21]	[1,22]

the sensor surface. So, although technically not an SPR-based biosensor, it is label free and that is all that really matters. Like an ELISA, the system is designed for end-point measurements. A rapid detection system tracks analyte binding in each well to yield screening and/or affinity information, with the entire plate being read within fifteen seconds. And because the BIND technology is based on standard microtiter plate formats, both its sampling and data output can be easily integrated with other robotic systems within the drug discovery pipeline. We suggest you check their website for more information on system availability.

Rumor has it that in early 2005 Biacore AB plans to release their next-generation SPR array chip platform (well, it is not actually a rumor – we read about this in their financial statement). They say this platform will incorporate ''increased throughput while maintaining the sensitivity of established Biacore technology''. It is unclear just how much of an increase in throughout we will see, but we look forward to the possibilities.

Comparison of technologies

As illustrated in Table 1, today's commercially available biosensors are complementary, not exactly competing, technologies. Each instrument fills a particular niche in drug discovery efforts. The versatile and well-established Biacore 2000 and 3000 are ideal for characterizing targets or developing new assays. Biacore S51 is poised to make a significant impact in secondary screening and lead optimization. Once researchers see that you can detect the binding of low-molecular-mass compounds to immobilized macromolecular targets, the S51 will become a ''must have'' technology to support all screening groups. GxP-compliant systems and software have expanded the realm of biosensors into areas where machines can better cope with highly repetitive tasks and data tracking. The 8500 Affinity Chip Analyzer is the first real-time, high-density protein array system and it is adding a whole new dimension to biosensor applications. Part of the challenge here is to develop the reagents and assays to take advantage of what the technology offers, but it is always good to have technology drive new applications. Paved roads came only about with the advent of the automobile. We are confident that in time, simultaneous experiments on hundreds of targets will be as routine as the two or three targets we are accustomed to working with now.

Conclusions

Producing high-quality commercial SPR biosensor instruments is actually challenging. It requires robust detectors, robotics, and fluidics, as well as handy surface chemistries, efficient software, and reliable customer support. But even the best biosensors in the world are missing one key component: the reagents (see Outstanding issues). A successful biosensor experiment depends on the user providing

Links

- Association of Biomolecular Resource Facilities: http://www.abrf.org
- BioLogic Software: http://www.biologic.com.au
- Biomolecular Interaction Technologies Center: http://www.bitc.unh.edu
- Biosensor Tools: http://www.biosensortools.com
- Center for Biomolecular Interaction Analysis: http://www.cores.utah.edu/interaction
- Ferrari: http://www.ferrari.it

good-quality biomolecules. We need to remember that biosensor technology, unlike mass spectrometry or UV–vis spectroscopy, requires that the reagents be active. Many of the skeptics of biosensor technology have often tried one experiment in the past and did not get the results they would hoped for. Their lack of success was probably due to poor reagents and/or poor experimental design than to any facet of the biosensor itself. So do not blame the messenger. The overwhelming majority of misinterpreted data and experimental artifacts arise from operator error, inadequate data processing, and often from fanciful data analysis. It is interesting to see the number of groups that, when they encounter a complex binding profile, jump to the conclusion that it is the result of some biologically interesting phenomena and fail to consider more mundane but probable causes such as sample heterogeneity, aggregation, or nonspecific binding. When biosensor experiments are carefully performed, response data can fit to a simple model and the standard deviation of rate constants from multiple users can be very low (<30%), which in fact demonstrates the reliability of biosensor experiments [5].

People who love biosensors can profit by learning how to use the instruments they already have in-house in a better way and by adopting new applications. In the biosensor community, the art of experimental design and data analysis is the, and is constantly evolving.

The new, application-specific biosensor platforms can minimize bottlenecks in the small-molecule and biopharmaceutical drug discovery process – it is time we take a serious look at what they can offer. SPR assays can rapidly confirm or refute hits from high-throughput screens and provide information to evaluate whether or not compounds should advance to the next stage of investigation. In lead optimization, the benefit is that structure/activity studies become more meaningful when affinity data is resolved into kinetics. This enables us to differentiate between compounds in a way that was not possible until now. Also, the interest in high-throughput screening systems and the proteomics revolution is driving the development of higher-capacity SPR biosensors. Time will tell what roles these emerging systems will adopt in the drug discovery process, but it is great to see companies like Biacore AB, Applied Biosystems and HTS Biosystems, and SRU Biosystems responding to our need for speed.

Related articles

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Outstanding issues

- Scientific community hesitates to accept SPR-based interaction studies even though the biosensor has been validated as a biophysical tool.
- Advances in biosensor instrumentation and applications are outpacing user skills. To take full advantage of this technology, users need significant training in experimental design and data interpretation.
- Biosensor experiments require high-quality reagents. When a biosensor experiment fails, it is most often due to a reagent's low activity, high nonspecific binding, poor stability, and/or low solubility.
- SPR array platforms present a new level of technical challenges, including how to spot or immobilize ligands and how to process large data sets efficiently.

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