Review

Survey of the year 2005 commercial optical biosensor literature

Rebecca L. Rich and David G. Myszka*

Center for Biomolecular Interaction Analysis, University of Utah, Salt Lake City, UT, USA

We identified 1113 articles (103 reviews, 1010 primary research articles) published in 2005 that describe experiments performed using commercially available optical biosensors. While this number of publications is impressive, we find that the quality of the biosensor work in these articles is often pretty poor. It is a little disappointing that there appears to be only a small set of researchers who know how to properly perform, analyze, and present biosensor data. To help focus the field, we spotlight work published by 10 research groups that exemplify the quality of data one should expect to see from a biosensor experiment. Also, in an effort to raise awareness of the common problems in the biosensor field, we provide side-by-side examples of good and bad data sets from the 2005 literature. Copyright © 2006 John Wiley & Sons, Ltd.

Keywords: affinity; Biacore; biomolecular interaction analysis; evanescent wave; IAsys; kinetics; optical biosensor; resonant mirror; Spreeta; surface plasmon resonance

Received 29 August 2006; accepted 30 August 2006

INTRODUCTION

Our review of the 2005 literature revealed the optical biosensor field is certainly not stagnant. Instead, the release of new commercial biosensor platforms, the development of novel assays, and the technology’s application in diverse biological applications continue to increase steadily. We found 1113 articles published in 2005 that describe the use of commercial optical biosensors to monitor binding interactions. For this review we considered optical biosensors that are based on surface plasmon resonance (SPR) or evanescent wave technology and monitor interactions in real time to provide kinetic rate constants. We did not include any articles dealing with nanoparticles and SPR. We felt that nanoparticles were better left for others to review.

Having read over 1000 articles, we found that what stands out the most in the literature is that there are a lot of biosensor users who have an inadequate understanding of the technology. Perhaps even more frightening is that the reviewers accept manuscripts containing low-quality biosensor data and journal editors are willing to publish them. We have said it before and we say it again here: the standard tenents of the scientific method seem to be forgotten when people start using biosensors.

In past reviews, we have used simulated data sets to illustrate common problems we see in the biosensor literature. This year we have chosen to include published data sets to stress the need to improve the quality of biosensor work. We highlight common errors in experimental design, as well as in data processing and analysis, which unfortunately are extremely prevalent in the literature.

Some people will argue that we are expecting too much from users. Is it too much to expect to see figures of experimental data with fits to the model when the authors report rate constants for an interaction? If you don’t show it, we don’t believe it and neither should the reader. Also, is it too much to expect to see replicate experiments performed? This is something we were taught to do in basic science classes in middle school (as we recall, it was two of the five ‘Rs’—reading, writing, arithmetic, replicate, and randomize). Reporting biosensor work should not be: ‘Here’s a single injection of some heterogeneous analyte over a surface with too high of a density of a randomly coupled ligand at too slow of a flow rate that we fit with a complex model which we don’t really understand but we report rate constants (with no standard errors) anyway.’

But before we get too far into what is wrong with the biosensor literature, we start with what is right. There are a small number of users who do produce excellent work. We have selected examples from the best of these to demonstrate the latest advances in biosensor applications and technology, as well as the quality of data we expect to see in screening, kinetic, and/or equilibrium analyses. These highlighted articles should be used as role models for other biosensor users to follow.

Then we hit you with the bad stuff. We use examples of questionable data sets published in 2005 to illustrate several recurrent problems in the biosensor literature. The purpose of including bad data is to educate biosensor users so that they can better interpret their own data, as well as evaluate the quality of what they see in the literature. If one of your data sets is highlighted as an example of poor-quality data, don’t feel too bad—rest assured you’re not alone.
REVIEWS, METHODS, AND THEORY

References 1–97 in this survey review various aspects of optical biosensors and their applications, while References 98–103 include detailed methods for the biosensor-based characterization of various biological systems and/or discuss the technology from a theoretical viewpoint. In the reference list, the reviews are subdivided into three groups: articles that focus on optical biosensor technology [1–19], articles that compare optical biosensors and other interaction technologies [20–54], and articles that illustrate how optical biosensors, coupled with other technologies, have advanced specific fields of research [55–97].

From a biosensor user’s perspective, several of these reviews are particularly noteworthy. References 4, 10, 11, 13, and 18 describe the SPR phenomenon, compare and contrast the different platforms and sensor surfaces that are available, and outline how optical biosensors are implemented in studying a range of interactions. Our surveys of the 2003 and 2004 literature highlight recently published work that demonstrates the wealth of information available from well-designed and properly executed biosensor experiments [15,16].

Other reviews provide detailed examples of the diversity of applications for optical biosensors [1,5,8,9,12,17]. O’Kennedy et al. [12] described the technology’s use for detecting microorganisms in water and food; Aguilar and Small [1] summarized the biosensor’s recent contribution to understanding β-amyloid interactions and fibril formation related to Alzheimer’s disease; Huber [8] outlined a biosensor-based strategy to streamline lead optimization in drug discovery; Swanson [17] demonstrated how the biosensor can be used in the development of therapeutic antibodies; and Choulier et al. [5] and Lin et al. [9] highlighted the biosensor’s impact in characterizing viral proteins and small molecule/oligonucleotide interactions, respectively.

In addition to providing an overview of SPR principles, instruments, and applications, Simpson and Fisher’s [102] article includes a step-by-step protocol for the SPR analysis of an antibody/antigen interaction; these methods can be incorporated into the study of other systems so their article serves as an excellent reference for the general biosensor user. Also, Gesellchen et al.’s [99] and Hartmann-Peterson and Gordon’s [100] protocols for characterizing protein interactions include tips for ligand immobilization, surface regeneration, analyte analysis, and data processing.

OVERVIEW OF PRIMARY RESEARCH ARTICLES

In 2005, investigators published results obtained from 31 different optical biosensors produced by the 18 manufacturers listed in Table 1; the overwhelming majority (87%) employed Biacore technology. Also, among the 881 articles describing Biacore-based studies, 84% included information about which platform was used: most often studies were performed using the 3000 (38%), 2000 (22%), X (16%), and 1000 (4%) platforms, with the residual 4% using S51 [157,757,760,761,768,779–783,789–791,918], Flexchip (formerly Applied Biosystems 8500 Affinity Analyzer) [138,338,434,435,583], Q [926,928,929,931,933,934,936,939,940], J [199,240,614,827,915], and Bialite [873].

Since Biacore instruments continue to dominate the optical biosensor field, articles describing Biacore work are subdivided by biological area in the reference list. As in the years past, the technology was used primarily to characterize antibody/antigen [294–445], receptor/ligand [446–580], and other protein/protein interactions [104–293], but we are seeing a steady increase in its implementation in small molecule [750–800], food/agriculture [926–940], clinical [941–955], and polymer [956–967] applications.

Table 1. Commercial optical biosensor technologies

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Platforms</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biacore AB</td>
<td>Biacore, Bialite, J, Q, X, 1000, 2000, 3000, Flexchip, S51</td>
<td>104–985</td>
</tr>
<tr>
<td>Affinity Sensors</td>
<td>IAsys, IAsys+, IAsys Auto</td>
<td>986–1025</td>
</tr>
<tr>
<td>Texas Instruments</td>
<td>Spreeta</td>
<td>378, 379, 709, 1026–1042</td>
</tr>
<tr>
<td>EcoChemie</td>
<td>Autolab Esprit</td>
<td>1043–1052</td>
</tr>
<tr>
<td>Optrel</td>
<td>Multiskop</td>
<td>1053–1062</td>
</tr>
<tr>
<td>GWC Technologies</td>
<td>SPRmager II</td>
<td>1063–1070</td>
</tr>
<tr>
<td>Nippon Laser &amp; Electronics</td>
<td>SPR-670</td>
<td>1071–1078</td>
</tr>
<tr>
<td>Windsor Scientific</td>
<td>IBIS, IBIS-II</td>
<td>1079–1086</td>
</tr>
<tr>
<td>Resonant Probes</td>
<td>SPRTM</td>
<td>1087–1092</td>
</tr>
<tr>
<td>Corning</td>
<td>Epic</td>
<td>1093–1096</td>
</tr>
<tr>
<td>Toyobo</td>
<td>MultiSPRinter</td>
<td>699, 718, 1097–1100</td>
</tr>
<tr>
<td>Analytical μ-systems</td>
<td>BIOSUPLAR, BIOSUPLAR-2</td>
<td>1101–1103</td>
</tr>
<tr>
<td>Reichert Analytical Instruments</td>
<td>SR7000</td>
<td>1104–1106</td>
</tr>
<tr>
<td>DKK Corp.</td>
<td>SPR-20</td>
<td>1107,1108</td>
</tr>
<tr>
<td>Genoptics</td>
<td>SPRI-Array</td>
<td>1109,1110</td>
</tr>
<tr>
<td>Artificial Sensing</td>
<td>OWLS</td>
<td>1111</td>
</tr>
<tr>
<td>Johnson &amp; Johnson Clinical</td>
<td>Unnamed</td>
<td>1112</td>
</tr>
<tr>
<td>Diagnostics</td>
<td>VirChip</td>
<td>1113</td>
</tr>
</tbody>
</table>

Copyright © 2006 John Wiley & Sons, Ltd.

DOI: 10.1002/jmr
Also, in 71% of the 2005 literature biosensors were used to determine kinetic and affinity parameters, 25% of investigations were qualitative (screening analytes to identify and/or rank binders), and 4% of users developed assays to detect/quantitate specific components of complex solutions.

**BEST-DRESSED ARTICLES FROM 2005**

From the 2005 literature, we highlight articles from 10 research groups that we recommend every biosensor user read. These articles exemplify today’s state-of-the-art applications in biosensor studies and range from well-executed, qualitative [184,294], kinetic [429,475,664,704,776,779,906,907], and equilibrium analyses [664,751,906,907] to the use of novel assay designs [526] and new technology [583]. Even more importantly, these articles illustrate the quality of data and interpretation that we expect to see in biosensor-based studies.

Jiang et al. [184] examined how wild-type and mutant versions of Hsc70 bound to immobilized auxilin in the presence of ATP. Using the biosensor in a screening format, the researchers determined how individual residues within the interface between the N-terminal nucleotide binding domain (NBD) and the C-terminal protein substrate binding domain (SBD), as well as within the linker region, contribute to this chaperone/chaperone interaction. Figure 1A displays a subset of the sensorgrams obtained from the comparison of 25 Hsc70 constructs: the mutants’ effects on auxilin recognition are clearly discernable (relative binding levels for the entire mutant panel are summarized in Figure 1B). From this study, Jiang et al. identified the interdomain interaction necessary for chaperone function and provided evidence for an allosteric binding mechanism.

Since the authors presented figures of biosensor data (i.e., Figure 1A), we can evaluate the quality of their experiment and interpretation. For example, the shapes of the response indicate the biosensor was properly maintained, the data were appropriately processed, and the interactions were well behaved (note how the shapes of the curves are exponentials). Although the response intensities for the best binders are larger (~1800 RU) than we usually recommend, in this qualitative screen these binding levels are acceptable since a relatively high auxilin surface density is required to observe differences between the weakest binders. Also, this study illustrates how the biosensor can be used to efficiently acquire information for a large set of interactions.

Figure 2 illustrates how Aldaz-Carroll et al. [294] used the biosensor to screen antibodies against the vaccinia virus membrane protein L1R. To identify each antibody’s binding epitope in the protein, a panel of antibodies was tested in a three-step approach (Figure 2A): (1) capture of L1R on a surface coated with anti-His antibody, (2) binding of the primary antibody, and (3) binding of the secondary antibody. As shown in Figure 2B, each of the eight anti-L1R antibodies used in this study recognized the captured protein. Figure 2C depicts the responses obtained when one of the antibodies (VMC-2) was flowed over various Ab/L1R pairs. VMC-2 binding was inhibited by VMC-3, -5, and -6, suggesting that these four antibodies bind to an overlapping epitope. The panel of interactions is summarized in Figure 2D and E: these data indicate three distinct antigenic sites exist on L1R. These studies, along with this research group’s mapping study of the antibodies targeting for the vaccinia virus extracellular protein B5R [295], demonstrate how the biosensor can be applied to the first few steps in the selection of antibodies to treat and prevent vaccinia-related diseases. The figures of biosensor data published by...
Aldaz-Carroll et al. in References 294 and 295 again demonstrate the importance of showing binding responses upon inspection, we can immediately identify competing antibodies, as well as critique the authors’ experimental design and execution.

From a technical viewpoint, Figure 3A, which shows the binding responses for the wild-type protein/oligonucleotide interaction, sets the standard for what we like to see in a figure of biosensor data. RNA was captured at a low density, resulting in low binding responses, and the wide range of protein concentrations produced concentration-dependent responses that ranged from almost baseline signals to nearly saturating the RNA surface. Each protein sample, as well as buffer blanks, was tested twice; the overlay of the replicates indicates the chosen regeneration condition was appropriate and the analysis was reproducible. Association was monitored long enough to produce curvature in the responses for the highest concentrations and dissociation was monitored long enough to observe decay in these signals. Finally, the figure includes the fit of a 1:1 interaction model to this set of binding responses. The overlay of the fit and responses demonstrates these binding events are well described by this simple model. In Figure 3B, the responses for the panel of mutants are all shown on the same scale. In Figure 3C, the responses for the panel of mutants are all shown on the same scale.

Using the biosensor to characterize the RNA recognition motif in U1A, a spliceosomal protein, Law et al. [704] compared the kinetics of mutant and wild-type U1A protein binding to biotinylated U1hpII RNA captured on streptavidin-coated flow cell surfaces. As shown in Figure 3, this analysis revealed how individual residues in the protein’s RNA-binding site contributed to the interaction.

Figure 2. Biosensor-based epitope mapping of the vaccinia virus L1R membrane protein. (A) Cartoon of the capture steps in each binding cycle: step 1, L1R capture on an anti-His antibody surface; step 2, antibody binding to L1R; step 3, another antibody binding to a different epitope in L1R. (B) Responses from step 2 for eight anti-L1R antibodies binding to L1R. (C) Responses from step 3 for VMC-2 binding to L1R after blocking with the other antibodies. VMC-2 binding in the absence of another antibody is labeled ‘control’ and corresponds to 100% binding; VMC-2 binding after blocking with VMC-2 is labeled ‘self’ and represents background. (D and E) Summary of the antibody/L1R interactions. In the table, black boxes highlight antibody blocking (>50%) interactions and white boxes indicate limited or no blocking. In the diagram, the three groups of blocking interactions between antibodies are segregated and blocking is indicated by a black line. Reproduced from Reference 294 with permission from Elsevier, Inc. © 2005.
same scale, which makes it easy for the reader to evaluate how each mutation altered the protein/RNA interaction. Figure 3C summarizes these kinetic parameters. We find that a graphical depiction of kinetics (like that shown in Figure 3C) is much easier to interpret than a simple listing of the rate constants.


Recent work performed by Karaveg et al. [906, 907] provides more examples of well-performed, well-presented biosensor experiments. These researchers used the biosensor to characterize the catalytic mechanism in endoplasmic reticulum mannosidase I, examining how a non-hydrolyzable Man9GlcNAc2 peptide substrate analog and a small-molecule inhibitor (dMNJ) bound to the immobilized enzyme (both wild-type and mutants). As illustrated by the 12 data sets in Figure 4, the peptide and inhibitor bind the enzyme via distinct mechanisms and the individual alanine substitutions in the enzyme produce a wide range of kinetic effects for both ligands.

With even a quick glance at Figure 4, readers can judge the quality of the data obtained by Karaveg et al. For example, these researchers designed the experiments so that, whenever possible, the range of tested analyte concentrations produced responses that ranged from nearly saturating the enzyme surface to concentrations that produce almost baseline responses (e.g., peptide binding to the T688A mutant). Also, the double-referenced binding profiles are of low intensities (as we expect for small analytes), are concentration dependent, appear to be describable by simple exponentials, and show curvature in the association phase and signal decay in the dissociation phase. Finally, the duplicate analyses of each analyte concentration overlay.

Although we can estimate data quality to a significant degree simply by looking at a set of sensorgrams, the next level of scrutiny is to determine how well the proposed model fits the binding responses. As shown in Figure 4, kinetic data for each mutant that displayed peptide or inhibitor binding were globally fit to 1:1 interaction models. Once again, a good overlay of the fit and data demonstrates it is possible to fit SPR data to a simple interaction model when the experiments are performed properly. Karaveg et al. took the analysis one step further: when appropriate, data sets were also fit using equilibrium analyses (shown in Figure 4 for peptide binding to wild-type, E599Q, and F659A enzymes). For each of these interactions, the responses for each analyte concentration reached a plateau during the association phase so these responses could be plotted against concentration and fit to a simple binding isotherm to obtain $K_D$. The agreement between the affinities determined from both the kinetic and equilibrium methods supports the use of a simple model to describe each interaction.

In addition to being a technically sound biosensor analysis, Karaveg et al.’s work established this diverse enzyme family’s requirements for substrate recognition and specificity. Also, the rate constants measured for the inhibitor/enzyme pairs provide fundamental information for developing next-generation inhibitors of class I mannosidases, a potential target for human protein misfolding disorders.


Towards understanding the mechanism of antibody-mediated neutralization of HIV-1 and SIV (simian immunodeficiency virus), Cole and coworkers resolved the kinetics of a panel of antibodies known to bind and neutralize variant SIVs interacting with soluble envelope trimers (gp140) [429]. These researchers’ intent was to determine if specificity and quantitative properties of antibody binding to SIV-envelope proteins correlate with neutralization. The mAbs were captured on protein A surfaces and tested for binding to soluble trimer. At the end of each binding cycle, the antibody/trimer complex was stripped from the protein A surface and a fresh aliquot of antibody was captured. Using the Biacore 3000’s four flow cells, three antibodies were examined in parallel with the fourth protein A-coated surface serving as a reference.

Figure 5A shows Steckbeck et al.’s initial characterization of gp140 binding to three mAbs. This analysis compared (1) the protein binding to a neutralizing mAb that recognizes a linear-epitope (3.11H, top panels), a conformation-recognizing neutralizing mAb (1.10A, middle panels), and a conformation-recognizing non-neutralizing mAb (3.8E, bottom panels), and as well as (2) the binding parameters obtained at 25°C (the temperature commonly used in antibody/antigen studies, left panels) and those obtained under more in vivo conditions (37°C, right panels). Once again, the overlay of the replicates demonstrates the selected regeneration conditions were appropriate and the binding partners were stable. In addition, the overlay of the fitted model and binding responses reveal how well the reported rate constants describe the interactions. While the affinities determined at 25°C and 37°C were similar, the $k_a$ and $k_d$ both increased at the higher temperature. This analysis emphasizes the importance of carefully selecting the analysis temperature when comparing and interpreting reaction rate constants.

Continuing the antibody characterizations at 37°C, Steckbeck et al. examined gp140 from neutralization-sensitive and neutralization-resistant SIV strains binding to mAbs. The
Figure 4. Kinetic analyses of a Man$_9$GlcNAc$_2$-peptide substrate analog (left panels) and a small molecule inhibitor, dMNJ (1-deoxymannojirimycin; right panels) binding to wild-type and mutant mannosidase. Binding responses (color-coded by analyte concentration) are overlaid with the fit of a simple 1:1 interaction model (black lines). Selected equilibrium analysis of peptide/enzyme interactions are shown in the insets. Reproduced from References 906 and 907 with permission from the American Society for Biochemistry and Molecular Biology © 2005.
Kinetic analyses are shown in 5B and Figure 5C depicts correlation plots of kinetic parameters and antibody neutralization. No correlation between kinetics and neutralization capacity was observed since no significant differences were seen for a gp140 binding to the two classes of antibodies (Figure 5C, left panels). But, kinetic rates did correlate with the neutralization sensitivity of a particular SIV strain. Compared to gp140 from neutralization-sensitive SIV strains, the neutralization-resistant gp140 bound the panel of mAbs with faster $k_a$’s and $k_d$’s (Figure 5C, right panels). This study once again illustrates the importance of examining interaction kinetics rather than simply measuring $K_D$’s: with affinity information alone, the researchers could not discriminate between neutralization-sensitive and neutralization-resistant SIV strains.


Figure 5. Kinetic analyses of antibody/antigen interactions. (A) Wild-type SIVmac239 recombinant gp140 binding to three surface-tethered mAbs at 25°C (left) and 37°C (right). Replicate binding responses for each analyte concentration (shown in color) are overlaid with the fit of a simple 1:1 interaction model. Black lines (B) Responses and model fits for rgp140s from SIV/17E-CL (left) and SIVmac239 (right) binding to neutralizing (3.8E, 3.10A, 5.5B, 3B3) and non-neutralizing antibodies at 37°C. (C) Summary of kinetic constants. Left panel: SIV/17E-CL rgp140 binding to neutralizing (filled squares) and non-neutralizing antibodies (open squares). Right panel: neutralization-sensitive (filled squares) and neutralization-resistant (open circles) SIV rgp140 binding to the mAb panel. Reproduced from Reference 429 with permission from the American Society of Microbiology © 2005.

Nordin et al.’s [779] biosensor-based characterization of kinase binding by small-molecule inhibitors demonstrates the technology’s versatility in both assay development and interaction analysis. First, these researchers used eight kinases, representative of this protein family, to optimize immobilization conditions and the analysis buffer. Comparison of standard covalent amine coupling, covalent amine coupling with a protective specific binder, antibody capture, and antibody capture followed by covalent amine cross-linking revealed that the best immobilization method needed to be determined individually for each kinase. In contrast, for all of these eight kinases, Tris-based buffers proved more appropriate than the HEPES-based buffers commonly used in kinase enzyme activity studies. Then, Nordin et al. characterized various aspects of kinase activity/specificity, including the identification of inhibitors that bind at the ATP site and determination of inhibitor selectivity of different kinase isoforms, as well between activated and unactivated kinases. For example, Figure 6 depicts the kinetic resolution...
of inhibitors of cyclin-dependent kinase 2 (Figure 6A) and p38α (Figure 6B). In each panel, the compounds bind the kinase with similar affinities but display different kinetics (apparent from looking at the different binding profiles shown in the left panels, as well as the $k_a$ vs. $k_d$ plots shown on the right). This study of kinase inhibitors again emphasizes the importance of determining rate constants: kinetics provides information about the mechanism of binding which cannot be obtained by equilibrium analysis.


In the 2005 literature that showed kinetic data sets overlaid with a model, complex models were used to interpret the binding responses 20% of the time. All too often, the kinetic parameters obtained from these complicated models were used to justify atypical binding mechanisms. In our opinion, complex responses rarely represent intrinsic binding events. Instead, they are a product of artifacts that can be minimized with appropriate experimental design and data processing.

Giannetti et al.’s [475] biosensor-based characterization of human transferrin receptor 1 (TfR) binding to iron-loaded transferrin (Tf) however, is a nice example of applying a complex interaction model (bivalency) correctly. First, the bivalency of this system is well established: TfR is a homodimer and Tf is composed of two non-identical iron-binding domains. Second, some of the mutations and buffer conditions these authors investigated diminished the inherent complexity of the TfR/Tf interaction; these data sets could be described by a simple interaction model, thereby demonstrating these experiments were well executed.

Figure 7 shows the binding responses and fits (with residuals included in panel A) obtained for iron-loaded and apo Tf binding to wild-type and mutant TfR. Since these investigators provided non-biosensor-based confirmation
crystallographic characterization) of a complex interaction and included examples of 1:1 interactions as ‘quality controls’ (bracketed by the red lines in Figure 7B), we are confident of their data interpretation. From these studies, Giannetti et al. identified residues in both TfR and Tf that are critical for iron release but distant from the iron binding site in Tf, which demonstrates that TfR transmits long-range conformational changes.

**Figure 7.** Kinetic analyses of ligand/receptor interactions. (A) Iron-loaded transferrin (Tf) and apo-Tf binding to immobilized wild-type and mutant transferrin receptor (TfR). Responses (black lines) for each analyte concentration are overlaid with the fit of a bivalent ligand model (red lines) and residuals are shown below each panel. (B) Responses and model fits for a panel of iron-loaded and apo-Tf proteins binding to wild-type and mutant TfR at a range of pHs. The data sets bracketed by the red lines were fit to a simple 1:1 interaction model; the other sets were fit to a bivalent ligand model. Reproduced from Reference 475 with permission from Elsevier Ltd. © 2005.


In an effort to tackle parasites that target DNA, as well as to understand molecular mechanisms of DNA recognition, Wilson and coworkers are developing new compounds that bind specific DNA sequences


Much of the work published by these researchers requires fitting to complex models, but without fail they perform supporting experiments to justify the complexity they observe in some systems. Figure 8 highlights a few of their recent biosensor-based investigations of small molecule/oligonucleotide interactions.

The kinetic analysis in Figure 8A illustrates how so-called linear and curved compounds bind differently to a DNA minor groove. While traditionally the design of minor groove binders has been based on optimizing compound curvature and functional group position, the data shown in Figure 8A indicate this linear compound binds with unexpectedly high affinity and suggest that some linear compounds may twist to fit in the groove. From a biosensor user’s perspective, the data shown in Figure 8A are exemplary. Miao et al. [776] tested a 100-fold range in analyte concentration, monitored the association and dissociation phases long enough to observe significant curvature in the responses, and perhaps most importantly, overlaid the data with the fit of a simple interaction model.

Figure 8B shows Bailly et al.’s [751] equilibrium analyses of ethidium, an intercalating agent that displays little preference for specific sequences, and DB950, a compound that forms minor groove complexes preferentially with

[664, 751, 776]. Much of the work published by these researchers requires fitting to complex models, but without fail they perform supporting experiments to justify the complexity they observe in some systems. Figure 8 highlights a few of their recent biosensor-based investigations of small molecule/oligonucleotide interactions.

The kinetic analysis in Figure 8A illustrates how so-called linear and curved compounds bind differently to a DNA minor groove. While traditionally the design of minor groove binders has been based on optimizing compound curvature and functional group position, the data shown in Figure 8A indicate this linear compound binds with unexpectedly high affinity and suggest that some linear compounds may twist to fit in the groove. From a biosensor user’s perspective, the data shown in Figure 8A are exemplary. Miao et al. [776] tested a 100-fold range in analyte concentration, monitored the association and dissociation phases long enough to observe significant curvature in the responses, and perhaps most importantly, overlaid the data with the fit of a simple interaction model.

Figure 8B shows Bailly et al.’s [751] equilibrium analyses of ethidium, an intercalating agent that displays little preference for specific sequences, and DB950, a compound that forms minor groove complexes preferentially with
were aliquotted and tested sequentially for antibody binding. Cells was lysed and centrifuged and then the supernatants of rigorously controlling the mechanics of receptor solubilization. The left panel in Figure 9A shows the panel of solubilization conditions efficiently to assess how a wide range of solubilization conditions affect conformationally sensitive antibodies revealed the CCR5 fraction that remained active in CHS/DOM/CHAPS- and Triton X-100-containing buffers. Figure 9C summarizes how a range of detergent/lipid/cholesterol combinations affected CCR5 activity. The optimal buffer condition from this screen was used to characterize how the two receptors bound native protein partners and small-molecule inhibitors. The top panel in Figure 9D shows this biosensor-based auto-solubilization method yielded CCR5 that specifically bound gp120 in the presence of CD4. In addition, the required CD4:gp120 ratio, as well as the receptor’s affinity for 1:1 CD4:gp120, could be determined (Figure 9D, middle and bottom panels).

In this analysis, Navratilova et al. demonstrated the power of the biosensor to provide information that can be used to overcome two significant hurdles that currently hinder the crystallization of membrane receptors. First, the activity/stability information obtained using the biosensor can be applied in assays (e.g., crystallization trials) that require active material but, up to now, have been hit-and-miss in identifying conditions that do not destroy the receptor. Second, tracking the activity of CXCR4 and CCR5 in parallel revealed that optimal buffer conditions are not universal for this class of receptors. Instead, ideal conditions must be determined empirically for each. From an even wider perspective, although this assay was initially designed for membrane receptors, it can serve as a template for developing efficient biosensor-based methods to optimize activity for a wide range of targets.


The Biacore Flexchip (formerly 8500 Affinity Chip Analyzer from Applied Biosystems) was the first array-based optical biosensor released worldwide. This instrument can simultaneously track up to 400 interactions in real time to provide kinetic information. Binding responses are recorded as analyte is simultaneously flowed across a matrix (up to 20 × 20) of ligand spots prepared in the single macro (~1 cm²) flow cell. Demonstrating the Flexchip’s efficient sampling throughput (as well as providing details regarding the Flexchip’s novel detection system and chip design features), Baggio et al. [583] mapped the contacts required for MEM-265 antibody binding to MHC class II protein HLA-DR1.

Using a panel of 18 Ala mutant peptides, these investigators spotted each biotinylated peptide at nine

AT-rich sequences, binding to a DNA hairpin duplex containing a single AATT site. The right panel, showing the fits of the equilibrium responses to binding isotherms, indicated DB950 binds more than 10-fold tighter than ethidium to the hairpin sequence. To determine these affinities, the DB950 binding responses required fitting with a complex model (compound binding at two sites in the DNA with different $K_D$’s). Since Wilson and coworkers collected responses for some interactions that could fit to a simple model and used alternative methods (i.e., electric linear and circular dichroism and fluorescence spectroscopy) to complement their biosensor analyses, we can be confident that there is a biological basis for the complexity observed in the DB950/hairpin interaction.

Buchmueller et al. [664] applied both kinetic and equilibrium analyses to investigate how pyrrole compounds target specific DNA sequences. In the example shown in Figure 8C, the compound displayed an affinity for TGGC hairpin almost 100 times higher than for CGCG and TCGA hairpins. Again, the interactions could not be described well by a simple 1:1 model. Instead, the responses were fit by a two-nonequivalent-binding-sites model.

Together, the data in these figures (and other articles published by Wilson and coworkers in 2005 [665,726, 753,787]) show how the biosensor can be used in both kinetic and equilibrium formats for genome-focused drug discovery. In addition to developing new drugs against DNA-targeting parasites, this research group consistently demonstrates the power of the biosensor to provide information that can be used to overcome two significant hurdles that currently hinder the crystallization of membrane receptors. First, the activity/stability information obtained using the biosensor can be applied in assays (e.g., crystallization trials) that require active material but, up to now, have been hit-and-miss in identifying conditions that do not destroy the receptor. Second, tracking the activity of CXCR4 and CCR5 in parallel revealed that optimal buffer conditions are not universal for this class of receptors. Instead, ideal conditions must be determined empirically for each. From an even wider perspective, although this assay was initially designed for membrane receptors, it can serve as a template for developing efficient biosensor-based methods to optimize activity for a wide range of targets.


Although membrane-associated receptors are extremely popular drug targets, their characterizations have been hampered by the difficulties involved in maintaining the proteins’ functions once they have been extracted from their native membrane environments. Using CXCR4 and CCR5 as representative members of this protein family, Navratilova et al. [526] developed automated biosensor-based methods to evaluate how a wide range of solubilization conditions affect receptor stability and activity. In these analyses, a tagged receptor preparation is (1) captured by immobilized mAb that recognizes the tag, (2) tested for binding to antibodies (both conformational and linear-epitope recognizing) or CD4:gp120 complexes, and (3) stripped from the mAb surface so that a fresh aliquot of receptor could be captured and tested. This approach made it possible to screen a large panel of solubilization conditions efficiently to assess how each condition affected receptor activity.

Figure 9B shows how the two receptors bound native protein partners and small-molecule inhibitors. Figure 8C, the compound displayed an affinity for TGCA target specific DNA sequences. In the example shown in Figure 9B, the compound displayed an affinity for TGCA hairpin almost 100 times higher than for CGCG and TCGA hairpins. Again, the interactions could not be described well by a simple 1:1 model. Instead, the responses were fit by a two-nonequivalent-binding-sites model.
positions (three spots each from peptide stock solutions of 1, 2, and 3 μM) on a NeutrAvidin-coated chip (Figure 10A). With buffer alone spotted at 18 positions to serve as negative controls, this spotting method produced a 12 × 15 matrix of spots that was tested at one time for antibody binding (Figure 10B). Figure 10C shows a subset of the responses obtained from one injection of antibody across the peptide matrix. Responses from the nine D66A spots (triplicates from the three peptide concentrations) demonstrated spotting was reproducible: (1) spot density was uniform since responses from triplicates spotted from the same peptide stock solution overlay and (2) the response intensities correlate with the concentration of stock peptide concentrations. Furthermore, the quality of the data is apparent in that these nine traces could be globally fit to a simple 1:1 interaction model to obtain kinetic parameters (Figure 10D). The responses shown in Figure 10E for antibody binding to two peptides spotted at similar concentrations illustrate that binding differences between mutants were readily apparent. Spots for six peptides showed little or no signal, indicating Ala substitutions in these peptides knocked out antibody recognition. In contrast, antibody binding to spots of the other 12 peptides could be characterized kinetically. The graphical summary of rate constants in Figure 10F indicates the affinities for antibody binding to these 12 mutants are due primarily to a difference in the dissociation rate constant: $k_d$’s varied by less than 3-fold but the $k_d$’s varied by about 48-fold. With this kinetic profiling, Baggio et al. identified a structural component in the antigen epitope recognized by MEM-265.

For the general biosensor audience, this work illustrated the Flexchip’s ability to yield binding parameters for an entire mutant panel from a single analyte injection, which increases throughput and reduces sample consumption. In addition, the Flexchip user does not need to worry about batch-to-batch variability in analyte preparations or the stability of the ligand surface over the course of a long-term analysis. While its drawbacks include the need to optimize

---

**Figure 9.** Using the biosensor to optimize activity of solubilized membrane-associated receptors. (A) Effect of post-solubilization wait time on receptor activity. Left: Antibody binding responses for four aliquots of CXCR4 that were captured on the chip surface 10, 20, 30, and 40 min after manual cell lysis. Right: Antibody binding responses for four aliquots of CXCR4 that, using the biosensor automation, were solubilized from whole cells, captured from the cell lysate, and screened for activity. (B) Comparison of antibody binding responses for CCR5 solubilized using CHS/DOM/CHAPS (left) and Triton X-100 (right). N-terminus-specific antibodies are shown in orange and blue; the other antibodies recognize conformational epitopes. (C) Screen of 34 CCR5 solubilization conditions. (D) Native ligand binding to solubilized receptor. Top: Responses for gp120 binding to CCR5 in the absence and presence of the co-receptor CD4. Middle: Responses at equilibrium plotted against CD4:gp120 concentration (main panel) and binding responses (inset) for varying ratios of CD4:gp120 binding to CCR5. Bottom: Responses (main panel) and equilibrium analysis (inset) for 1:1 gp120:CD4 binding to CCR5. Reproduced from Reference 526 with permission from Elsevier, Inc. © 2005.
spotting conditions and small-molecule sensitivity limitations, the Flexchip is a promising technology for kinetically screening panels of antibodies and other proteins, as well as peptides and oligonucleotides.

WHAT NOT TO PUBLISH

In the television program What Not to Wear\textsuperscript{TM}, a contestant is first confronted with her/his poor wardrobe choices (often with a painful degree of frankness), but then the show’s hosts describe how the contestant should apply fashion basics. Each episode ends with the contestant incorporating the hosts’ fashion recommendations, although sometimes begrudgingly. In this section, we apply What Not to Wear’s tough-love approach to biosensor analysis. We start by showing data sets of questionable integrity (alongside examples of high-quality data sets), we then outline how to fix several of the problems that frequently appear in the biosensor literature, and, finally, we leave the reader to make the required adjustments to their own experiments.

Box 1: What do the sensorgrams look like?

- Where are the data?
- Are the curves exponentials?
- Are the response intensities reasonable?

When we read a article that contains biosensor data, we evaluate the reliability of the authors’ conclusions based on the 10 criteria described in Boxes 1–3. First, by answering

Figure 10. Kinetic mapping of an MHC peptide epitope using Flexchip. (A) Image of the assembled Flexchip chip, with the matrix of spotted peptides apparent within the macro flow cell. (B) 12 × 15 matrix of biotinylated peptides spotted on NeutrAvidin-coated chip, with the nine D66A peptide spots (triplicates of 1, 2, and 3 \( \mu \)M) highlighted. Regions between the spots were used for referencing. (C) Affinity traces for antibody MEM-265 (25 nM) binding to the nine D66A peptide spots. The box encloses the data region used in kinetic evaluation. (D) Affinity traces for the MEM-265/D66A interaction overlaid with the fit of a simple 1:1 model. (E) Overlay of MEM-265 binding to P56A and E52A peptides spotted at similar densities. (F) Kinetic screening summary for 12 peptides. Diagonal lines represent \( K_D \) values. Adapted from Reference 583 with permission from the American Society for Biochemistry and Molecular Biology © 2005.
the intensities of the binding responses. As illustrated in ligand, we also judge an experiment’s reliability based on mass of the analyte as well as the density of immobilized churned out of their machine as a reflection of the true instrument cleaning and/or servicing. This brings up another dispersion in the sample plug and may be eliminated by incorrect referencing, while the dips in response at the end of cannot be described by binding events. Instead, the response the unusual responses shown in Figure 12D and E, which and C. Now that you know what to look for, it is easy to spot examples of good binding profiles are shown in Figure 12B kinetics but have the same affinity. Additional real-life exponential profiles change for interactions that differ in depicts three simulated data sets that illustrate how the association phase, and (3) decreased as analyte dissociated increased as analyte bound to the ligand surface during the responses (1) were stable before the analyte injection, (2) highlight in Figures 1–10. In each of these figures, the examples of exponential profiles are shown in the work we binding signal may not be a cell but instead may be fractions of membrane or solublized protein or other junk. This brings up another good point to consider: what you assume you inject may not actually be what is responsible for the binding responses. How well do you know your sample? Since the biosensor is most often used to determine kinetic and affinity constants, Box 2 describes the criteria we use to evaluate the experimental design of published data for quantitative studies.

Box 2: How was the experiment designed?
- Is there curvature in the association phase?
- Is there decay in the dissociation phase?
- Do analyte concentrations span a wide range?

For kinetic studies, the analyte injection should be long enough to produce curvature in the association phase (for at least the highest analyte concentrations) and the dissociation phase should be monitored long enough to observe decay in the signal. Always remember that curvature is required for a fitting program to output a unique set of rate constants; linear responses in the association phase can often be described by varying ratios of and Figure 14A and B illustrate that extending the analyte injection for longer than the typical 0.5–1 min, (6 min in Figure 14A), as well as monitoring the dissociation long enough to see signal decay (9 min in Figure 14A), provided enough information to fit these responses to reliable binding parameters (the data and fit are overlaid in Figure 14A). In contrast, the analyte injections shown in Figure 14C and D are too short. By increasing the injection times, curvature would be apparent and the data sets could be rigorously fit to obtain reliable rate constants. Also, responses in the dissociation phases in Figure 14C and D show little to no decay so these data would be fit equally well by any slower than \(10^{-3} \text{s}^{-1}\). As a rule of thumb, monitoring the dissociation phase until we can visually detect decay in the signal provides enough information to fit...
the data to obtain a unique $k_d$. Furthermore, replicate analyses would be required to show this slow decay is reproducible and that the baseline is stable over the same period of time.

All too frequently we see experiments that would be improved if more, particularly lower, analyte concentrations were included. (We are particularly puzzled by instances in which only high analyte concentrations are studied—testing more dilute analyte concentrations would require comparatively little sample.) In both kinetic and equilibrium analyses, responses from a wide range of concentrations are required to obtain robust binding constants. In the examples shown in Figure 15A and B, the tested analyte concentrations produced responses ranging from almost baseline intensities to saturating (Figure 15A) or nearly saturating (Figure 15B) the ligand surface; these data sets

---

**Figure 11.** Scaling binding responses. (A and B) Responses scaled to emphasize the binding events. In panel B, the two panels are scaled similarly to illustrate the differences in response intensities. Reproduced from References 127 and 489 with permission from the American Society for Biochemistry and Molecular Biology © 2005 and The American Society of Hematology © 2005. (C and D) Responses that are scaled to emphasize the spikes that bracket the analyte injections. Reproduced from references 305 and 896 with permission from Walter de Gruyter © 2005 and the American Society for Biochemistry and Molecular Biology © 2005.
could be fit to obtain reliable rate and affinity constants. In the kinetic analysis (Figure 15A), responses from the high analyte concentrations determine the ligand’s binding capacity and responses from low analyte concentrations contribute to the calculation of $k_a$ and $k_d$ and help define the mechanism. In the equilibrium analysis (Figure 15B), data points from the nine concentrations are used to define the binding isotherm precisely. The kinetic and equilibrium analyses shown in Figure 15C and D, respectively, would be improved if lower analyte concentrations (and, for the example in Figure 15D, higher concentrations as well) had been included. In Figure 15C, the individual responses from the narrow range of analyte concentrations provide the fitting algorithm with similar information and in Figure 15D the responses from these three concentrations do not provide enough information to accurately define the affinity of this interaction.

Figures of data also reveal how well binding responses were processed, analyzed, and interpreted. By answering the questions listed in Box 3, we can determine if the reported kinetic and equilibrium constants are valid.

**Box 3: How were the data processed/analyzed?**

- Are the responses appropriately referenced?
- In equilibrium analyses, does each response achieve a plateau in the association phase?
- Are kinetic data overlaid with the model fit?
- Is a bulk-shift correction applied appropriately?
For example, binding responses with small signals (<50 RU) should first be double referenced (corrected for the response from a reference surface as well as for the response from buffer blanks) prior to data fitting. The small spikes at the start and end of the injections, as well as the overall exponential profiles of the responses, in Figure 16A and B indicate these data sets have been referenced. In contrast, insufficient referencing in Figure 16C and D is indicated by the large shifts in response at the start and end of the analyte injections. Unfortunately, these jumps are too often erroneously attributed to a significant binding event instead of being recognized as a result of mismatches in refractive index between the sample and running buffer.

In equilibrium analyses, the response for each analyte concentration must reach a plateau (which indicates equilibrium is achieved) during the association phase. Figure 17A and B show excellent examples of performing an equilibrium analysis properly. Figure 17C and D also demonstrate the importance of showing the overlaid data and fits: in these examples, the data are not well described by the model so we know to consider the reported rate constants as estimates at best. But, only 20% of kinetic analyses published in 2005 included at least one figure of data overlaid with the model fit. When no fits are shown, we are left to guess if the fitting was done appropriately and whether or not the reported rate constants approximate the binding responses. Figure 18C and D depict two kinetic data sets that, in the actual published figure, were not overlaid with model fits. Shown as red lines in Figure 18E and F are the fits we simulated using the rate constants the authors listed in tables that accompanied their data sets. The problem is that the rate constants they report do not match the experimental data. This is a fairly common occurrence in the literature and makes us wonder how valid any reported rate constants are when the authors fail to show the fits.
Figure 14. Choosing lengths of association and dissociation phases. (A and B) Analyte was injected long enough to observe curvature in the association phase and decay in the dissociation phase. The responses in (A) are overlaid with the fit of a 1:1 interaction model. Adapted from References 585 and 852 with permission from the American Society for Microbiology © 2005 and the Society for Endocrinology © 2005. (C and D) Linear association-phase responses for the entire set of analyte concentrations and dissociation-phase responses that show no decay. Reproduced from references 497 and 466 with permission from Elsevier Ltd. © 2005 and the American Society for Microbiology © 2005.

Figure 15. Choosing the analyte concentration range. (A and B) Responses obtained from a wide range of analyte concentrations in kinetic (A) and equilibrium (B) analyses. The responses in (A) are overlaid with the fit of a 1:1 interaction model. Adapted from References 390 and 895 with permission from the Nature Publishing Group © 2005 and the American Chemical Society © 2005. (C and D) Narrow ranges of analyte concentrations used in kinetic (C) and equilibrium (D) analyses. Panels (C) and (D) reproduced from references 877 and 487 with permission from Elsevier, Inc. © 2005.
Even after data are properly referenced, there will sometimes be small bulk shifts during the association phase when samples and running buffers have very different refractive indices. These vertical jumps (or dips) in response (highlighted in Figure 19A and B) can be accounted for when fitting data to a kinetic model. However, this bulk-shift correction should be applied judiciously. For example, it should not be used to mask heterogeneity in the interaction as was done in Figure 19C and D. Nor can it be used to characterize responses obtained from a sub-optimally maintained instrument. The unusually shaped responses in Figure 19E and F were most likely the result of instruments that needed servicing or samples that contained aggregates.

We found numerous examples in 2005 in which users floated the bulk-shift parameter in the kinetic analysis software and this resulted in complete misinterpretation of the binding events. For example, in Figure 19G the authors float the bulk shift and end up reporting an $R_{\text{max}}$ of 1.48 RU for this data set, which, if true, would represent less than 3% of the total signal they show. The rate constants that they report from this analysis would yield the simulated responses shown in the inset. Clearly, the shapes of the data do not relate to the recorded binding events.

The data shown in Figure 19H are a concern for several reasons. First, these are responses generated for a $\sim$13 kDa protein binding to 1000 RU of an immobilized $\sim$70 kDa protein, so based on the mass ratio they should have seen maximum responses of around 200 RU. Instead, the responses are less than 10% of what we expect ($<12$ RU), indicating that the proteins may be inactive or may actually not interact. Second, the authors apply a bulk-shift correction to these data, which results in an even lower $R_{\text{max}}$ for their putative interaction (about 2 RU, 1% of the expected response), and from their kinetic fitting report a $k_d$ of $1 \times 10^{-5}$ s$^{-1}$ and a $K_D$ of 11 nM. What these investigators have done is to describe the majority of the binding data with a bulk shift and then to ascribe a high-affinity interaction to a signal of about 1.5 RU (note the tiny signals and fitted lines during the dissociation phase). The inset in Figure 19H depicts the responses we simulated from their reported rate constants. Again, the binding responses are not at all described by their reported rate constants. If these authors have measured an interaction with a $K_D$ of 11 nM, they did it using a surface that is $<1$% active. How valid can these results be? And how does this level of analysis get published? This data set epitomizes many of the concerns we have about the biosensor literature.

**SUMMARY**

It is wonderful to see optical biosensor technology applied in so many research areas, but the pool of good biosensor data published in 2005 is disappointingly small. We estimate that only 10% of the literature can be considered reliable. The remainder either included no figures of data or presented data indicating additional experimental optimization was required and/or the responses were incorrectly interpreted.
Figure 17. Fitting equilibrium responses. (A and B) Responses at equilibrium (highlighted by the dashed boxes in the left panels) fit to simple binding isotherms and Scatchard analyses (right panels) to obtain interaction affinities. Binding responses also fit using a Scatchard analysis are shown in the inset of panel (A). Reproduced from References 728 and 117 with permission from Elsevier, Inc. and the American Chemical Society © 2005. (C and D) Responses that do not reach equilibrium during the association phase fit to a binding isotherm (C) and a Scatchard analysis (D). The arrow in panel C shows the isotherm that corresponds to the set of binding responses (the other isotherm was obtained for a different interaction). Reproduced from references 461 and 677 with permission from the American Chemical Society © 2005 and Wiley VCH-Verlag GmbH & Co. © 2005.
By showing good and bad data sets we hope to teach biosensor users and the greater scientific community how to evaluate biosensor data. For this comparison we picked examples almost at random; those we selected are by no means the only problematic data we found.

The problem of poor-quality biosensor experiments is not new, but in order to fix the problem, the first step is to acknowledge that a problem exists. Second is to determine where this problem is coming from. We sometimes hear people say that the instrument manufacturers are to blame since they produce machines that are too easy to use. That is like trying to blame automobile manufacturers for accidents caused by drunk drivers. No, the real problem lies with the instrument operators.

It is clear that the typical biosensor user does not understand the fundamentals of the technology. Also, we are not talking about the physics of SPR—we are talking about the fundamentals of biological molecules. A single band on

---

**Figure 18.** Overlaid kinetic responses and model fits. (A and B) Responses that are well described by the model fits (shown in red). Reproduced from References 679 and 467 with permission from the American Society for Biochemistry and Molecular Biology © 2005 and the American Chemical Society © 2005. (C and D) Responses that are not well described by the model fits, but from which rate constants were reported. Reproduced from references 974 and 417 with permission from Elsevier, Inc. © 2005 and IWA Publishing © 2005. (E and F) Responses (black lines) from which rate constants were reported but fits were not included in the original figures. Binding profiles simulated from the reported rate constants and analyte concentrations are shown overlaid in red. Reproduced from references 922 and 925 with permission from the Japanese Pharmacological Society © and Elsevier B.V. © 2005.
Figure 19. Applying bulk-shift correction. (A and B) Appropriately applied bulk-shift correction. The slight adjustments at the beginning and end of the analyte injections are highlighted by red dashed ovals. In both data sets, the responses are overlaid with the fit of a 1:1 interaction model (black lines). Reproduced from references 787 and 392 with permission from the American Chemical Society © 2005 and Elsevier, Inc. © 2005. (C and D) Bulk-shift correction applied to heterogeneous data sets. In both data sets, the responses are overlaid with the fit of a 1:1 interaction model (black lines). Reproduced from references 534 and 210 with permission from Oxford University Press © 2005 and the Federation of European Biochemical Societies © 2005. (E and F) Bulk-shift correction applied to responses that are indicative of instrument and/or problems. Reproduced from references 822 and 152 with permission from the American Chemical Society © 2005. (G and H) Bulk-shift correction applied to square-shaped responses. Red responses shown in the insets depict binding profiles simulated from the reported rate constants and analyte concentrations. Reproduced from references 800 and 656 with permission from Elsevier, Inc. © 2005.
an SDS–PAGE gel doesn’t tell you anything about the conformational purity or the activity of a sample. Whoever coined the adage ‘garbage in, garbage out’ must have been referring to biosensor work. Compounding the issue is the fact that many biosensor users do not know why a sensorgram should look like an exponential, or even what an exponential looks like. Most could not explain the difference between a first-order and second-order binding event. These are basic kinetic concepts that have either never been taught or have long been forgotten in the world of molecular biology kits. Well, until biosensor instrument manufacturers go so far as to develop the proteins that we need for all our analyses, there is still some fundamental biochemistry that is left up to the user. That is where the problem lies.

Our deepest concern about the quality of biosensor work is rooted in two issues. First is that the number and types of biosensor instruments are rapidly evolving. We are seeing new high-end instruments that increase the sample throughput and numerous lower-budget units that will increase the user base. We worry that if the quality of the work does not improve, these advances will simply lead to even more bad data. Second, as systems biology becomes tractable there is an opportunity to extract information from biosensors to plug into interaction pathways. Unfortunately, the reliability of much of the kinetic information in the literature is—how should we say it?—unreliable.

Yet we remain optimistic. Optical biosensors are incredibly versatile devices. When applied correctly, this technology provides information that is critical to basic research and drug discovery. We should all do our part to see the work is done right. Also, by the way, don’t wear plaid with stripes.

REFERENCES

Reviews

Optical biosensors


Interaction technologies


Methods and Theory


Biocore

Proteins


DOI: 10.1002/jmr


Copyright © 2006 John Wiley & Sons, Ltd.


DOI: 10.1002/jmr


Antibodies


Receptors


Journal of Molecular Recognition 2006; 19: 478–534

DOI: 10.1002/jmr

513 SURVEY OF THE YEAR 2005


Copyright © 2006 John Wiley & Sons, Ltd.


DOI: 10.1002/jmr

Copyright © 2006 John Wiley & Sons, Ltd.


actions with a surface F1-ATPase-related structure and apolipoprotein A-I. Immunity 22: 80–89.


Oligonucleotides


                  deficiency virus type 1 blocks gp120-CCR5 interaction. J. Virol. 79: 13806–13810.


                  Chem. 6: 123–132.


                  Res. 33: 2166–2175.


686. Halder K, Chowdhury S. 2005. Kinetic resolution of biomo-
                  lecular hybridization versus intramolecular folding in nucleic acids by surface plasmom resonance: application to G-quadruplex/duplex competition in human c-myc pro-

                  sensitive element of human c-myc promoter: Cto T mutation in C-rich strand enhances duplex association. Biochim. Bio-

                  SURVEY OF THE YEAR 2005 521


                  dance detection of a DNA point mutation by polymeric microspheres. E-Polymers no. 060.


698. Kojima T, Takey O, Ohtsu M, Kawarasaki Y, Yamane T, Nakano H. 2005. PCR amplification from single DNA mol-
                  ecules on magnetic beads in emulsion: application for high-throughput screening of transcription factor targets. Nucleic Acids Res. 33: e150.


702. Kojima T, Takey O, Ohtsu M, Kawarasaki Y, Yamane T, Nakano H. 2005. PCR amplification from single DNA mol-
                  ecules on magnetic beads in emulsion: application for high-throughput screening of transcription factor targets. Nucleic Acids Res. 33: e150.


Small molecules


minor groove binders: induced fit interactions of heterocyclic dications with the DNA minor groove. Biochemistry 34: 4840–4843.


Extracellular matrix


Carbohydrates


Clinical support


935. Himmelberg S, Aranha L, Damen JMA, Sliper M, Schellekens H, Crommelin DJA, Jiskoot W. 2005. Structural character-


Cells, phage, and virus particles


Copyright © 2006 John Wiley & Sons, Ltd.

DOI: 10.1002/jmr


Other applications


Affinity Sensors


### Texas Instruments


Copyright © 2006 John Wiley & Sons, Ltd.

**J. Mol. Recognit.** 2006; 19: 478–534

DOI: 10.1002/jmr
EcoChemie


Optrel


GWC Technologies


Nippon Laser & Electronics


Windsor Scientific


Resonant Probes


Corning


Toyobo


Analytical μ-systems


Reichert Analytical Instruments


**DKK Corp.**


**Genoptics**


**Artificial Sensing**


**Johnson & Johnson Clinical Diagnostics**


**Vir Biosensor**