Survey of the year 2007 commercial optical biosensor literature

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In 2007, 1179 papers were published that involved the application of optical biosensors. Reported developments in instrument hardware, assay design, and immobilization chemistry continue to improve the technology’s throughput, sensitivity, and utility. Compared to recent years, the widest range of platforms, both traditional format and array-based, were used. However, as in the past, we found a disappointingly low percentage of well-executed experiments and thoughtful data interpretation. We are alarmed by the high frequency of suboptimal data and over-interpreted results in the literature. Fortunately, learning to visually recognize good—and more importantly, bad—data is easy. Using examples from the literature, we outline several features of biosensor responses that indicate experimental artifacts versus actual binding events. Our goal is to have everyone, from benchtop scientists to project managers and manuscript reviewers, become astute judges of biosensor results using nothing more than their eyes. Copyright © 2008 John Wiley & Sons, Ltd.

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INTRODUCTION

Legendary New York Yankees’ catcher Yogi Berra said “You can observe a lot by just watching.” This is particularly true for optical biosensor studies. The best way to get a sense for the overall field of optical biosensor research is simply to look at the data presented in the literature. When you do this, as we have done every year for the past twelve years (totaling almost 10,000 papers), you quickly realize that the biosensor community can be stratified into two layers and we need to get a new hobby.

The top layer is populated by the “haves.” These biosensor users have a good understanding of what it takes to set up, execute, and interpret an experiment. The bottom layer consists of the “have-nots.” These users do not have a clue how to properly use biosensor technology. They regularly employ poor experimental design and are quick to apply complex models and overinterpret their data. Unfortunately, the bottom layer is much thicker than the top; think cupcake. Based on the literature, we estimate that only about 15% of users are in the icing. Fortunately, the numbers of good users are growing, albeit too gradually compared to the expansion of the total biosensor user base. It is like someone added way too much yeast to this cake.

What keeps us up at night is the fact that poor-quality data gives all biosensor technology, and even the good users, a black eye. With the junk we see presented in the literature it is not surprising that biosensor data often do not agree with results from other methods (or even with other biosensor measurements). At best, this frustrates the scientific community. At worst, they are ready to throw the baby, nanny, and the whole tub out with the bathwater.

If you are wondering if you belong in the icing or the cake, try answering these three questions: (1) what shape should a binding response be (and why)? (2) How are the shapes of association and dissociation phases related? (3) What is your favorite color? Prepare yourself to find the answers to these fundamental questions and more as we present our review of the 2007 literature.

OVERVIEW OF THE YEAR’S LITERATURE

Keep your eye on the ball—Ford Frick

We found 1179 papers published in 2007 that demonstrate the wide range of optical biosensor applications. These papers, listed in the Reference section, are divided into reviews of optical biosensors (1–40), generally applicable descriptions of the theory behind the technology (41–43), and recent developments in immobilization chemistries and assay design (44–78), and research papers that focus on a specific biological system (79–1179). We apologize in advance if we missed your paper; next time, add surface plasmon resonance ( SPR), biosensor, or interaction analysis as keywords. You can also e-mail us a PDF or, better yet, mail us a hard copy of your paper with a 20-dollar bill attached to it so we know to file it with the good ones.

Books, chapters, and review papers

Every year we find a fairly large number of book chapters and review papers that outline how optical biosensors work and the technology’s utility in specific applications. (We look forward to the day when good research papers out-number reviews.) In the Reference list, the reviews are subdivided into those that focus exclusively on biosensor technology (1–10) highlight the technology’s contributions in various applications (11–20), and describe the impact of biosensors, along with other technologies, in a particular field of biology (21–40).

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Summarizing the state of SPR instrumentation today, Schasfoort and Tudos’ *Handbook of Surface Plasmon Resonance* is a good read (particularly the Foreword) (8). Author contributions include descriptions of the theory and history of SPR technology, the variety of available instruments and surface chemistries, and the basics of measuring kinetic and affinity parameters. Specific chapters provide in-depth discussions of the biosensor’s contribution in food and clinical analyses, the development of SPR imaging platforms, and the future of this technology.

More specific in their focus, several review papers provide an introduction to optical biosensors (2), outline various methods to tether ligands to sensor surfaces (9) and describe recent developments in the technology (3,5,10). In our survey of the 2006, biosensor literature we highlighted ten papers which demonstrated the capabilities of biosensors (7). In addition, we used figures of data culled from the literature to discuss, and hopefully dispel, a number of myths that surround the technology. We and others also discussed where the technology is heading with regards to improved throughput and sensitivity (1,4,6) the advent of several array-based SPR platforms is one area to keep your eye on.

References 11–20 demonstrate the expanding roles biosensors are playing in drug discovery (11) and biodetection in medicine, food and the environment (12,20), as well as their general applicability in characterizing proteins (18) and oligonucleotides (13,14,16,17). Majka and Speck’s, Nguyen et al’s, and Redman’s descriptions of SPR’s utility in nucleic acid research are particularly noteworthy (14,16,17). They included critical details about preparing oligonucleotide surfaces and testing proteins and small molecules as analytes, but more significantly, these authors provided background on the principles of SPR, as well as guidelines for experimental design and data analysis, that are applicable in general.

References (21–40) illustrate how the biosensor complements other investigative techniques in characterizing biological interactions, including carbohydrates (32) oligonucleotides (33,35,39) and traditional protein systems (31). Har discussed the technology’s contributions to drug discovery (28) while several authors highlighted its growing impact in medical diagnostics (21,30,40) environment monitoring (23,36,40) and food analysis (24,25,27). From a more technical standpoint, Berggard et al. summarized many of the methods used to characterize proteins (22) Hansen described novel approaches to prepare protein-coated surfaces (29) and Nice et al. did a nice job of detailing the steps involved in, and the value of, using biosensors in affinity purification (34).

Theory and method development

Edwards, Foley et al., and Hu et al. described theoretical elements of biosensors, including aspects of analyte delivery to, and dynamics of binding at, the ligand surface (41–43), while a number of authors developed unique immobilization strategies (44–62) and/or outlined novel assays (63–78).

Immobilization approaches

In 2007, slightly more than half of the authors covalently linked the ligand to the biosensor surface, with more than 90% of them using amine-coupling chemistry. While amine chemistry produces viable surfaces for a wide range of proteins, it can introduce surface heterogeneity and, in some cases, can inactivate the ligand. To better maintain ligand activity after coupling, various groups employed alternative methods to produce homogenous, active ligand (e.g., antibody (45,49,50) protein (46,47,55) and DNA (44)) surfaces.

Tagged ligands can also be tethered on the surface through capturing agents; commonly this is done through streptavidin or capturing antibodies. But capturing methods also have drawbacks: for example, the inability to strip biotinylated ligands from streptavidin surfaces or the gradual dissociation of captured His$_x$-tagged ligands from anti-His antibody or nitroloacetic acid-derivatized surfaces. Several research teams reported developing mimics of the avidin/biotin system (48,58) or combining features of the His$_x$ tag and bacterin systems to produce reliable, regenerate-able, captured-ligand surfaces (57,61).

Assay designs

Most often, biosensors are used to determine kinetic and affinity parameters (70% of the time) or in a qualitative mode to identify and/or compare binding partners (25%). But, the technology is extremely versatile, as demonstrated by users’ creativity in developing assays to detect components of food/environmental samples (65,66,72,78) optimize protein production/purification (67,69,73) and rank antibodies in high throughput (71,76).

For example, Jacobsen et al. used the biosensor to identify an elution condition for the purification of urokinase-type plasminogen activator receptor (uPAR) (69). A sensor chip with antibody, inhibitor peptide, and native binding partner immobilized in individual flow cells mimicked three potential affinity matrices. The researchers tested uPAR binding to, and pH-dependent dissociation from, the surfaces to establish (1) which pH was appropriate for complete surface regeneration and (2) which ligand best maintained activity over repeated regeneration cycles. And finally, the team also used the biosensor to confirm the activity of the receptor preparation after purification.

Leonard et al. took the opposite approach. Without performing any pre-analysis purification steps, these researchers used the Biacore A100 platform to rapidly rank both the titer and kinetics of recombinant scFv antibody fragments in crude bacterial lysates (71). This work also demonstrated the parallel processing capabilities of the A100 with their assay conditions, Leonard et al. reported that they could screen 400 antibodies per day.

INSTRUMENTATION

I am eye. I am a mechanical eye. I, a machine, am showing you a world, the likes of which only I can see—Dziga Vertov

We sure live in an exciting time. It seems that every month or so we hear an announcement about the launch of new optical biosensor. Our first response is Great! but then we wonder, how will this affect the price of gold? And could more machines lead to more bad data? But let’s ignore that negative thought for a moment.

As of last count, there are more than 50 different sensor platforms either in production or under development. This explosion in the field is evidenced by the more than 1100 reports we found of research using 42 different biosensor platforms offered by 25 different manufacturers. To make the different systems easier to follow, the Reference list is divided by detection...
method and subdivided by manufacturer (the specific platforms used are included in each section heading).

Traditional-format SPR biosensors
Most of the 2007 literature (96%) described research performed using standard SPR and related technologies (79–1131) which typically can test up to three ligands at one time. We found work performed using instruments from fifteen different manufacturers—we sure have come a long way since the early 1990’s when Biacore was the only manufacturer. But, we still find most of the publications (89%) utilize Biacore technology (79–1015). Because of their large number, the Biacore-based papers are subdivided by biological application. While most researchers used the technology to characterize proteins (79–277), antibodies (278–451), receptors (452–596), peptides (597–692), and oligonucleotides (693–753), we continue to see it contributing to the characterization of small molecules (754–810), carbohydrates (811–859), lipids (860–899), and the extracellular matrix (900–935). In addition, Biacore biosensors are now more frequently used to study self-assembled monolayers and polymers (936–962), as well as membranes, viruses, and cells (963–975), and to identify components of crude samples in clinical support (976–987) and the food, agricultural, veterinary, and environmental sciences (988–995). And finally, while 75% of Biacore-based analyses involved the 2000 and 3000 platforms, 18% used the X or T100 (the remaining studies were performed with A100, X100, 1000, S51, C, J, Q, or BiAlite.)

SPR imaging (SPRi) biosensors
One rapidly developing technology is array biosensor platforms, which simultaneously monitor interactions across the entire surface of a large-format flow cell. Compared to standard SPR platforms, SPRi offers higher ligand throughput and flexibility in surface patterning. Once sensitivity and surface chemistry options are further developed, we see the application this biosensor design truly expanding. Toward these goals, researchers at Agilent reported developing a novel detection technology that significantly exceeds the sensitivity of other SPRi (and SPR) platforms (1132). In addition, they claim their detector could simultaneously monitor up to 110 ligand spots. Additional reports describing work using six other SPRi platforms are found in References 1133–1165.

Non-SPR optical biosensors
We often think that all optical biosensors employ gold surfaces to generate surface plasmons as the detection principle. But there are of course other optical detection methods: Axela’s dotLab involves a diffractive optics technology (1166), Corning’s Epic (1167–1169) and SRU Biosystems’ BIND (1170–1174) employ resonant waveguide grating, and Fairfield platforms are based on dual polarization interferometry (243,911,1175–1179).

THE SHAPE OF THE BINDING RESPONSE

It is necessary to keep one's compass in one's eyes and not in the hand, for the hands execute, but the eye judges — Michelangelo

For years now, we have written ad nauseam about proper experimental design and data analysis being the keys to generating high-quality sensor data. But we have come to realize is that the biggest problem with the biosensor user base is even more fundamental: the majority of users do not understand what a binding response should look like and they have no idea where the shape comes from.

We are not talking about needing to understand the quantum mechanics of plasmon generation. We are talking about the simple shape of a binding profile. The shape should be an exponential. Not parabolic, not hyperbolic, not concave in the association phase, and not convex in the dissociation phase. And certainly not all bumpy and wavy like we often see in the literature. It is an inconvenient truth that not every piece of data generated by an expensive piece of scientific equipment (e.g., optical biosensors) represents a true binding event. Responses due to mismatches in sample and running buffer, nonspecific binding, analyte aggregation/precipitation, poor instrument performance, and heterogeneity are too often misinterpreted as interesting biological events.

So before we jump into the literature, let’s take a minute to train our eyes how to recognize simple-exponential and non-exponential data. Take a look at the responses shown in Figure 1 panels A through E. Can you tell what they have in common? That’s right, they are all examples of simple binding interactions that conform to a single exponential. Sure, the data sets may look a little different compared to one another but that is because the rate constants are different. (In fact, the $k_d$ in panels A, B, and C are the same but the $k_d$ increases 100 fold across these three data sets. The changes in shape demonstrate how the $k_d$ affects the binding profile in both the injection and wash phases of the response.) Square-shaped responses, which result from a very fast $k_d$ (Figure 1D) can even be described by a simple exponential, as can responses that, at higher concentrations (e.g., in Figure 1E), begin to saturate the ligand surface. For all of these data sets, the fundamental mechanism of $A + B \rightarrow AB$ is valid. The simple exponential shape is what your eyes should be looking for when you view any sensor data, be it yours, ours or someone else’s.

Now what do you see when you look at Figure 1F? What you should see is that the initial binding rate appears linear rather than an exponential. This is an example of a binding reaction that is partially limited by mass transport. Mass transport limitations occur when the binding rate of an analyte to the ligand is faster than its diffusion rate to the surface. We have covered the subject numerous times in past publications so if you want more information just Google “mass transport and biosensors.”

What we want you to see is that mass transport introduces a linear component at the beginning of the injection phase and makes the dissociation phase non-exponential. Fortunately, interpreting mass transport-limited data is not a problem; the proper model just needs to be applied in data analysis. But this is a shape that you should be able to recognize by eye and know how to handle in terms of improving the experimental design (hint: use lower density surfaces and higher flow rates).

Now once the responses go beyond a simple exponential or a simple interaction limited by mass transport, the world can get very complicated. Figure 1G and H are examples of what people commonly refer to as biphasic binding responses. You will hear them say their data show they have a fast interaction and a slow interaction. While this may be true to some extent, the fact is that once you get biphasic data it
becomes virtually impossible to resolve what actual event is leading to the complex response. This is because biphasic data can be described equally well by a multitude of models. And if you do not know it by now you probably will not believe us when we say it here, but you cannot prove a mechanism is correct by modeling. Look it up in Wikipedia (we just entered it).

Finally, the last training sets for your eyes are Figure 1I and J. These are examples of drifty (Figure 1I) and spikey, jumpy, junky (Figure 1J) sensorgram shapes. To tell the truth, we see these sometimes in our own experiments. But we realize that these are caused by instrument artifacts, sample aggregation, or some other strange artifact we may not fully understand. When we see these responses, we take a step back and clean the instrument, prepare new reagents, and redesign the assay in order to improve the quality of the data.

In fact, a problem in most of the published data we see is that the authors apparently did only one experiment; it looks like they walked up to the machine, chucked in their samples, and published whatever data came out. Generating high-quality data is an art form and takes some effort. Oftentimes the initial experimental conditions, be it the immobilization chemistry, surface density, regeneration condition, and/or analyte concentration, have to be optimized. Many users who generate poor-quality data are either too ignorant to recognize the problem or too lazy to want to fix it. Even the artist Jackson Pollock put time and effort into his work; he was not just dripping paint on a canvas.

RECOMMENDED READING

_In my mind’s eye, I visualize how a particular… sight and feeling will appear on a print. If it excites me, there is a good chance it will make a good photograph. It is an intuitive sense, an ability that comes from a lot of practice—_Ansel Adams

Now that we have trained your eyes to recognize good and bad data, you are prepared to view with us the 2007 primary publications. To ease you into it, we have selected four examples,
highlighted in the grey box, that depict well-performed biosensor analyses. We recommend that every user model their experiments after these examples, which also include important details regarding experimental design and data analysis, as well as well-presented figures.

Figure 2 shows kinetic data sets published by Seet et al. (669), Keeler et al. (508), and Alexander-Brett and Fremont (453). In each panel, the effects of site-specific mutations (Figure 2A), pH (Figure 2B), and NaCl concentration (Figure 2C) are revealed by trends in the binding profiles. Even more importantly, the high quality of each experiment is apparent. The responses are reasonably shaped and concentration dependent. The responses of replicate analyte injections are superimposable, indicating the reagents were stable and the analysis was reproducible. And, the data are overlaid with the fit of a 1:1 interaction model, revealing how well the reported rate constants actually describe the observed binding events.

The fact that some people actually run replicate assays is comforting. Call us old-fashioned, but we think testing (and showing!) replicates should be mandatory. Just because biosensors are machines does not mean we can now stop being scientists. Rule #1: replicate and randomize.

In addition, the data in Figure 2 panels A and B are simple exponentials, whereas the data in Figure 2C are partially mass transport limited (like the example shown in Figure 1F). This mass transport contribution is most apparent in the responses obtained at 200 mM NaCl but decreases at higher salt concentrations. So even without fitting these data, we can tell that the association rate of the interaction slows down with increasing salt concentration. With these (and other) high-quality data, it is easy to evaluate the effects of different buffer conditions and/or mutations even before applying a fitting model.

Similarly, Figure 3 highlights a thorough equilibrium analysis of TCR and MHC mutants (470). In this figure, each response is
Figure 3. Highlighted equilibrium analysis: TCR-I binding to peptide-MHC-I surfaces. (A) In each panel, all responses reach equilibrium by the end of the injection phase ($t = 60$ s). Kinetic parameters listed in the insets were obtained via global fitting to a 1:1 interaction model. (B) Responses at equilibrium are fit to a simple binding isotherm to obtain the affinities reported in the insets. (The original paper does not include the responses corresponding to the isotherm shown in panel H.) Reproduced from Reference 470 with permission from the American Association of Immunologists Inc. © 2007.
clearly a simple exponential and reaches a plateau before the end of the analyte injection, which is required for an equilibrium analysis. In addition, the fit of each data set to a simple binding isotherm is included. Cole et al. also fit the responses in Figure 3A to a simple 1:1 kinetic model. (The fit and data overlay so well in the Figure that it is difficult to distinguish between the two.) As expected, the affinities determined from the equilibrium and kinetic analyses agree.

Figures 2 and 3 serve two purposes: (1) they demonstrate that it is possible to obtain experimental data that fit a simple interaction model as good as that we simulated for Figure 1, and (2) they set the standard for what published biosensor data should look like.

INTERPRETING RESPONSES

Beauty is in the eye of the beer holder—Kinky Friedman

It is surprisingly easy to produce a change in signal using the biosensor. It is not so easy, however, to produce responses that indicate actual binding events. Figure 4 shows data sets that all display simple-exponential profiles although their shapes vary widely (e.g., the responses in the wash phase of panels B and F decay much faster than those in panels D and E). As expected, the responses increase (and some level off) during the injection phase while they decay exponentially during the wash phase. Each of these data sets could be analyzed further to obtain meaningful binding information (even though in several panels fitting the data is not necessary to interpret the results).

But, unfortunately the examples in Figure 4 are not representative of the biosensor literature in general. In fact, a lot of poor-quality data (if you can even call it data) get published. Some examples of poor-quality data are shown in Figure 5. First, in several panels the responses decrease (some even go negative!) during the injection phase. (Before you jump to the conclusion that the negative response is due to some post-binding conformational change as many users do, realize that it’s likely a result of more nonspecific binding to the reference surface compared to the reaction surface.) Valid responses show an increase in signal as analyte binds to the ligand surface. Second, many of the responses are not smooth curves; these data sets contain jumps, dips, and wiggles. And third, the responses cannot be described by a simple exponential. Simply by looking at these responses it should be apparent that any response due to interesting interactions are at best complicated by—and, at worst, overwhelmed by—artifacts due to poor experimental design, reagent quality, instrument performance, and/or inadequate data processing. Yet we often find investigators trying to extract kinetic parameters from data like these.

Figure 5 also emphasizes the importance of showing data. With these figures, we know to regard the authors’ biosensor-based observations with suspicion. Without these figures, we would be left to wonder if they obtained reliable data. In 2007, 75% of papers describing biosensor work included at least one figure of sensorgrams. While this is a significant improvement over years past, we can only assume that the other 25% did not include figures because they were afraid to reveal just how bad their data were. A word to the wise: you should demand to see the data before believing anyone’s biosensor results.

EASILY IDENTIFIABLE ELEMENTS OF BINDING RESPONSES

The eye of the master will do more work than both his hands
—Benjamin Franklin

As we have been discussing, binding responses are information rich, much of which is apparent even before model fitting. For example, with a little practice you can estimate dissociation rates by eye. And, simply looking at profiles, you can determine if mass transport influences the interaction, the ligand surface capacity, and the appropriateness of using an equilibrium analysis, as we discuss below.
Mass transport contribution

We mentioned that mass transport limitations can be easy to spot by eye. Figure 6A shows four more examples of mass transport-limited data from the literature. You should be able to pick up the linearity in the responses. These authors correctly applied a mass transport limited model to extract estimates of the binding constants.

At this point we want to add a word of caution: sets of completely linear responses (e.g., Figure 6B) do not necessarily result from mass transport effects. Instead, these data may be a result of having a slow dissociation rate in combination with testing low analyte concentrations. To obtain reliable rate constants, it is important to collect responses that display some curvature. This may require longer contact times or higher analyte concentrations.

Saturation of ligand surface

Ligand saturation occurs when binding sites on the surface are fully occupied with analyte. Ligand saturation should not be confused with equilibrium binding responses (see below). We often see people mixing up these concepts. While a response that reaches saturation will be at equilibrium, an equilibrium response may not be at saturation.

Under conditions that approach saturation, the responses for a range of analyte concentrations (e.g., the three highest concentrations in Figure 1E) have the same (or nearly the same) intensity by the end of the injection phase. The intensity of the response produced when the ligand is saturated is the maximum response, $R_{\text{max}}$. This parameter can be used to determine the capacity of the surface, which in turn reveals the activity of the immobilized ligand as well as the stoichiometry of the ligand/analyte interaction.

Examples of data sets in which the higher analyte concentrations nearly saturate the ligand surface’s available binding sites are depicted in Figure 7A. Although the interactions have different profiles, in each panel the responses for the higher concentrations approach a maximum intensity. For each of these systems, going to even higher concentrations of analyte should not give a larger response because the ligand sites are saturated.

There are plenty of examples of biosensor experiments, however, in which saturation is not so obvious and $R_{\text{max}}$ is not so well defined. For example, responses in the left panel of Figure 7B increase well beyond what appears to be saturation of
an initial binding first site. At increasing concentration, binding occurs (with different kinetics) at a second site, which also begins to show saturation. Conversely, the responses in the right panel of Figure 7B were converging to saturation until very high analyte concentrations were tested. Sometimes, the response continues to increase (and may become more complex) as the analyte concentration increases. Most likely this is due to heterogeneity in the ligand, analyte, or both. Weakly binding material or higher levels of non-specific binding is often observed at higher analyte concentrations. Unfortunately, these secondary events are often misinterpreted as interesting binding events and researchers use complex models to describe these data.

**Figure 6.** Mass transport contribution. (A) Mass transport-influenced data sets. (B) Linear responses that are not necessarily a result of mass transport. Reproduced from References 61,260,450,753,795,832,968 with permission from Elsevier Inc., the American Chemical Society, the authors (Reference (832), Springer, and the Nature Publishing Group © 2007.

**Figure 7.** Ligand surface saturation. (A) Data sets in which the higher analyte concentrations nearly saturate the ligand surface. (B) Data sets that do not display definitive saturation. Reproduced from References 61,203,387,467,470,593,669,683,786 with permission from the American Association of Immunologists Inc., Rockefeller University Press, Elsevier Inc., Walter de Gruyter and John Wiley & Sons Ltd. © 2007.
Think about this: if an interaction has an intrinsic affinity of 1 nM, does it make sense biologically speaking to test for complex formation at 10 uM? Should we be surprised that we see some heterogeneity in a system when we test concentrations 10,000 times the $K_D$? And why stop there? Perhaps we should test it at 1 mM or higher. We work under the premise that it is best to characterize an interaction using concentrations around the $K_D$ whenever possible. The necessity to demonstrate saturation in a biosensor experiment is overrated and can lead you down the wrong path.

**Interaction equilibrium**

Biosensor analysis 101: an equilibrium binding response occurs when the same number of complexes forms as break down on the sensor surface. Therefore, the response will be flat at equilibrium. This leveling off (or plateau) in response is illustrated in Figure 1D. In this cartoon, the responses for each analyte concentration reach a plateau during the injection; therefore, these responses are all at equilibrium and the complete data set can be used in an equilibrium analysis to determine affinity. Data sets in which all responses reach equilibrium before the end of the injection, and therefore were appropriately used in equilibrium analyses, are shown in Figure 8A.

Maybe this seems obvious. But we are confounded by the number of groups who apply equilibrium analysis to data that have not reached equilibrium. Figure 8B shows four data sets inappropriately used in equilibrium analyses. In each example, the responses at the end of the injection phase were plotted against analyte concentration even though few, if any, of the responses reached equilibrium. This is a common mistake we see in the literature. *Equilibrium analysis is not “end-of-injection analysis.”* Do we need to write a software program that would not allow the user to fit data that has not reached equilibrium? It would be easier if biosensor users and journal reviewers would just learn the meaning of the word “equilibrium.”

To top it off, we often hear people complain that they get different affinities if they fit their data using kinetics versus an equilibrium analysis so something must be wrong with the biosensor. We hate to tell you this but it’s not the biosensor. The values are different because you carried out an equilibrium analysis using data that were not at equilibrium.

If equilibrium was not bad enough, let’s now talk about something that is easy for people to misunderstand...kinetics.

**ELEMENTS OF KINETIC DATA ANALYSIS**

*Living is easy with eyes closed, misunderstanding all you see*  
— John Lennon

As if obtaining interpretable data was not hard enough, a lot of people have trouble fitting kinetic data sets correctly. And, as the number and diversity of fitting options increase, it can be tough deciding which options to apply and when. Should I quit with a simple fit? When should I apply a bulk-shift correction? Do I even understand what I am doing when I click buttons in some software program?

**Show data overlaid with fit**

For years we have said that when you report kinetics, you must include a figure of data overlaid with the fit of the model. Why? Well, we just showed you that most users cannot properly perform an equilibrium analysis, so what makes you think they can perform a kinetic analysis right? All too often we see examples of producing poor-quality data and then using an inappropriate model without showing the fits. This gives us fits. We cannot trust that a study was performed properly without seeing fits to the data, so show both. Besides, adding a fit to the data does not take up any more space in a publication.

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**Figure 8.** Equilibrium analyses. (A) Responses that reach a plateau by the end of the analyte injection and can therefore be used in an equilibrium analysis. (B) Data sets that were used in an equilibrium analysis even though all responses did not reach a plateau. Reproduced from References 112,271,463,550,685,758,847 with permission from the American Association of Immunologists Inc., John Wiley & Sons Ltd., Elsevier Inc., the American Society of Hematology, and the American Society of Hematology © 2007.
Why you need to show the fit is illustrated in Figure 9. Since no fit was provided in the original publications, we simulated responses based on the authors’ reported rate constants. Just by looking at the figures you can see the reported rate constants do not describe the data very well. So we are left to wonder, how did these authors come up with these numbers?

Unfortunately, only 25% of authors reporting rate constants included figures of data sets overlaid with fitted model. In lieu of showing the fits, sometimes authors included residuals or $\chi^2$ values. Do not be fooled; clearly, they had something to hide. Only by seeing the data and fits overlaid can we really judge the quality of the results.

Red is one of the strongest colors, it’s blood, it has a power with the eye. That’s why traffic lights are red I guess, and stop signs as well... In fact, I use red in all of my paintings—Keith Haring

And, we are often asked "what is the best way to present biosensor data?” We prefer a combination of red lines (representing the model fit) overlaid atop black lines (the binding responses). Even though that’s not why we picked them, the examples shown in Figure 2 illustrate how easy this presentation style is on the eye. If we had our way, we would make this color combination mandatory.

Apply appropriate fitting models

Never bend your head. Always hold it high. Look the world straight in the eye—Helen Keller

Fitting should not be a subjective process but, unfortunately, it is often treated that way. Step 1 is to test if your data fit to a 1:1 interaction model (either with or without mass transport). The examples shown in Figure 10 once again demonstrate it is indeed possible to get biosensor data that fit well to a simple model for a variety of biological systems. Although, in some panels the data and fits do not overlay exactly because of a small amount of complexity in the data, we can feel fairly confident about the binding constants determined from these data sets. If the model does not fit, then Step 2 should be to go back and redesign the experiment to improve the quality of the data. Step 2 is not to start fitting with complex models.

The biphasic responses in Figure 11 are the most common shapes produced in biosensor experiments. Probably because the data were not perfectly described by 1:1 model, these authors tried more complicated fitting methods. But simply because it fits better does not mean it is correct. Of course the model will fit better when you add exponentials, but complex data can be fit equally well by a variety of models (e.g., heterogeneous ligand or analyte, conformational change, etc.) so the fit becomes meaningless. Where does it end? Why not use a mass transport limited-surface heterogeneity-conformational change model with a drifting baseline? (That’s a rhetorical question.)

Keep in mind that although biphasic shapes are common, they are not necessarily biologically relevant. Complexity in some of the data shown in Figure 11 is most likely due to using oligomeric analytes or conformationally impure ligand surfaces. We bet that if the authors try alternative immobilization methods, lower surface densities, and/or better quality reagents, the interactions will fit better to a simple model. We beg you to stop using complex models simply because they fit. We still have some clothes from high school that fit, but wearing them in public would be embarrassing.

Incorporating a bulk shift correction

Bulk-shift correction factors have been incorporated into biosensor data-processing software programs for some time now. This parameter, typically a correction of only a few RU,
Figure 10. Overlays of responses that are well described by the fit of a 1:1 interaction model. Reproduced from References 57,71,198,342,593,669,683,800 with permission from Elsevier Inc., Rockefeller University Press, the American Thoracic Society, John Wiley & Sons Ltd., and the American Chemical Society © 2007.

Figure 11. Kinetic data sets overlaid with fits of complex interaction models. Reproduced from References 299,397,549,609,728,872 with permission from the Nature Publishing Group, Elsevier Inc., Oxford Press, and the American Association for Cancer Research © 2007.
accounts for the square-shaped response that is due to a slight mismatch between sample and running buffer.

Several features of a data set indicate if the bulk shift correction can be applied. First, consider the proportion of the supposed bulk shift relative to the binding signal. The contribution from the bulk shift should be very small. Second, the bulk shift should be smaller at lower analyte concentrations as the analyte is diluted more with running buffer. And third, although it should be obvious, you cannot use this correction if the actual binding events also produce square-shaped responses. (For these systems, a more sophisticated calibration is required).

Figure 12A depicts a dataset in which using the bulk-shift correction was appropriate. The signal change due to the bulk shift is a small fraction of the total binding response and, as expected, the buffer mismatch (and therefore the correction) decreases with analyte concentration.

Unfortunately, we see the bulk-shift correction applied indiscrimately. Too often it is used as a surrogate for a complex model to mask complexity arising from suboptimal experimental design and/or non-homogeneous reagents. For example, the responses in Figure 12B, although clearly complex, were apparently fit using bulk shift as a make-shift correction factor. We see too many authors applying the bulk-shift corrections incorrectly, either out of ignorance or arrogance.

Figure 12. Applying the bulk-shift correction (A) appropriately to account for small buffer mismatch jumps and (B) inappropriately to mask complexity in binding. Reproduced from References 577,684 with permission from the American Society for Microbiology and Elsevier Inc. © 2007.

A CASE STUDY IN PUBLISHING BIOSENSOR DATA

You can turn your back on a person, but never turn your back on a drug, especially when it’s waving a razor sharp hunting knife in your eye—Hunter S. Thompson

The year 2007 marked a pivotal year for biosensor analysis with one group actively questioning the results of another in, of all journals, *Science*. Liu et al. published data allegedly showing the binding of a 42 kDa Gα protein subunit (GPA1) binding to a G protein-coupled receptor (GPCR) immobilized on the sensor surface (Figure 13A) (524). While studying G-protein interactions...
is not new in terms of biosensor analysis, what makes this interaction unique is that it would be the first example of a GPCR interaction in a plant! Indeed, if true, this would be worthy of publication in *Science*.

So let’s look at their data. The top panel in Figure 13A shows the responses for GPA1 injected across immobilized GCR2 and the bottom panel shows the same analytes over a bovine serum albumin surface, which served as a control. In the bottom of the figure, they report the rate constants for the interaction and an affinity of 2.1 nM.

When we look at these data the first thing we wonder is: why did not this group just subtract the reponse from the reference surface to correct for bulk refractive index changes and instrument drift? They used a Biacore 2000. Proper data referencing would reveal that if there is any binding response on the GCR2 surface above the reference, it is small (probably < 5 RU). Now while it’s true that we have been a strong proponent of keeping the surface capacity low, as a first demonstration of binding these levels are much too low. We are also proponents of demonstrating the response is reproducible and showing the fits to a simple interaction model, which this group failed to do.

In addition, Liu et al. report a dissociation rate of $3.9 \times 10^{-5} \text{s}^{-1}$, which corresponds to a half-life for the complex of ~5 h yet they only measure the dissociation phase for 3 min. Given their very low binding signal, it’s impossible to define such a slow dissociation rate in such a short period of time.

The best thing that we can say about Liu et al.’s study is that it prompted a *Comment* from Johnston et al. Recognizing the poor quality of the data and incongruity in the results, Johnston et al. simulated the responses expected for this interaction based on Liu et al.’s reported results, both without and with a contribution due to bulk shift (Figure 13B) (503). In their *Comment*, Johnston et al. also provide additional arguments as to why Liu et al.’s work is suspect. In their *Response* to Johnston et al.’s *Comment*, Liu et al. (525) supported their discovery with additional biosensor data which, frankly, is as poor quality as their original report. As a homework assignment, we will leave it to you to look over these publications and decide for yourself if the first plant GPCR has been discovered.

From our perspective, it is comforting to see other groups refuse to turn their back on poor-quality studies and instead stand up for the need for better work. One disadvantage of living in a democracy is that apparently anything goes in terms of execution and presentation of biosensor work. But an advantage of course is that we have the freedom to express our disbelief. And it is nice to see we are not alone.

**SUMMARY**

With a little practice one can identify biosensor responses that reflect plausible binding events, as well as those produced by artifacts. Recognizing good and bad biosensor data is in fact easy. Fixing the bad data is admittedly more challenging, but using complex models is only a crutch (your leg is still broken). The percentage of users who have a good understanding of how to use biosensor technology is unfortunately low but it is growing. We look forward to the day when we have to scour the literature to find an example of bad data. Right now it’s just too easy. And to make matters worse, we still find that a majority of users either do not show the data or do not show a fit to their data. If we were in charge, it would be a requirement that authors show data and fits when reporting binding constants.

*Let every eye negotiate for itself and trust no agent*

—William Shakespeare

**REFERENCES**

**Books, chapters, and review papers**

**Focus on technology**


**Biosensors in biology**


Focus on biology

Theory

Method development

Immobilization strategies
Assay design


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Antibodies


therapeutic IgG antibodies with three different N-linked Fc oligosaccharides: the high-mannose, hybrid, and complex types. Glycobiology 17: 104–118.


Optical bio Sensor literature.
In 2007, the field of optical biosensor literature continued to expand with significant contributions. A variety of researchers, including Iwema T, Billas IML, Beck Y, Bonneton F, Nierengarten H, Chaumot A, Hoos MD, Ahmed M, Smith SO, Van Nostrand WE, Hesp JR, Raven NDH, and others, published their findings. These studies often involved the development and application of novel optical biosensors, which have the potential to revolutionize diagnostics and drug discovery.

For instance, Edwards PD and colleagues investigated the development of a novel peptide that targets the HIV-1 gp41 core-binding motif, which is crucial for viral envelope assembly.

Another significant contribution came from Hansen RK, Christensen C, Korshunova I, Kriebel M, and others, who introduced a novel, cyclic amidine derivative that exhibited potent activity against enfuvirtide-resistant virus. This work highlights the importance of developing new strategies to combat drug-resistant strains of HIV.

In addition, the use of phage display technology was explored by several groups, including Kastning K, Kukhtina V, Kittler JT, and others, who identified novel short peptides isolated from a novel octamer peptide, CNSCWSKD, that induces fibril assembly by myelin basic protein. This work underscores the potential of phage display as a tool for the discovery of novel therapeutic peptides.

Furthermore, the use of structural and functional techniques, such as crystal structure studies, also continued to advance. For example, Lim CS and colleagues elucidated the structure of a specific antigen, demonstrating the potential of structure-guided design of peptidic ligands for human prostate specific antigen.

The use of artificial intelligence and machine learning in the design of biosensors also gained traction, with researchers like Lee E, Hayes DB, Langsetmo K, and others, employing computational methods to predict and design novel peptide inhibitors.

In summary, the year 2007 was marked by a flurry of activity in the optical biosensor field, with contributions ranging from the development of new peptides to the use of advanced computational methods. These advancements promise to further enhance the capabilities of optical biosensors in various applications, from disease diagnosis to drug discovery.


Oligonucleotides


Oligonucleotides


Small molecules


Carbohydrates


Lipids and micelles


Self-assembled monolayers, polymers, and films


Membranes, viruses, and cells


Clinical support


Other applications


Biosensing Instrument: Bis-SPR 1000


DKK-TOA: SPR-20


Ecochemie: ESPIRIT, SPRINGLE


Microvaccum: OWLS


Moritex: SPR 670 M, SPR-CELLIA


**NeoSensors: iAsys**


NTT-AT: Handy SPR


Optrel GB: Multiskop


Plasmonic Biosensor: Plasmonic


Reichert: SR7000


Resonant Probes: SPTM


Surface plasmon resonance imaging

Agilent


Biacore: Flexchip


BioRad: ProteOn XR36


Genoptics: SPRi-Plex


**Non-SPR-based optical technologies**

Axela: dotLab


**Coming: Epic**


**SRU Biosystems: BIND**


**Farfield: AnaLight 200, AnaLight Flex**


