Foreword

Make no bones about it, I love surface plasmon resonance (SPR)-based biosensor technology. After spending three years trying to measure binding constants using analytical affinity chromatography, I immediately saw the benefits of SPR the first time I sat down in front of a Biacore in 1991. Even today, no comparable technology exists to characterize molecular interactions in real time without labeling in an automated and robust fashion. But as the technology has expanded over the years, I find that there are three general attitudes towards SPR. There are the nay-sayers who hate the technology. There are long-time users who think they are experts. And there are the users who recognize they do not know everything about SPR but are eager to improve their skills.

Ever since the first commercially viable instrument was unleashed in 1990 by the biosensor group at Pharmacia (which was spun out into a separate company called Biacore in 1996, only to be acquired recently by General Electric, which previously bought Amersham who at one time had merged with Pharmacia, so in fact now the biosensor group has come full circle, even though they have always shared the same cafeteria in Uppsala, Sweden), there have been critics of SPR technology. So much so that in 2003 I created a character called "Dr. Evil Pessimist", who represents a composite of the various detractors of SPR. Dr. Pessimist rants and rages about problems he has with the technology, including nonspecific binding, instrument drift, mass transport and avidity effects. He argues that since SPR uses a surface the rate constants we measure will never reflect solution-based binding constants. In fact, much of his resentment of the technology stems from the fact that his experiments fail or his data never fit a simple model.

It has been my experience that there are two primary causes of this SPRa-phobia: poor-quality reagents and/or poor experimental design. Perhaps the molecules Dr. Pessimist is studying do not in fact interact or the preparations of samples are not active to begin with. Don't shoot the messenger. Dr. Pessimist asserts that his proteins are of high quality because they are "a single band on an SDS page gel". He fails to realize that this is not evidence of an active preparation or a conformationally homogeneous sample. I think biosensor experiments are akin to protein crystallography. No structural biologist I know would attempt to crystallize an impure, half-denatured preparation of protein

that has precipitated at the bottom of an Eppendorff tube. The sad thing is that garbage into a biosensor will often give complex responses that users misinterpret as some interesting binding event.

I have found that when experiments are designed appropriately with good-quality reagents and data are processed and analyzed properly, binding responses can be routinely fit to a simple interaction model. However, unlike Dr. Pessimist, I do not expect to obtain perfect binding responses when I set up experiments on a new interaction. I realize that obtaining high-quality data is an iterative process. In my research group, we usually set up a trial experiment to verify that the binding partners actually interact. Then we will often try different coupling chemistries, surface densities, and/or buffer conditions to optimize surface activity.

And when it comes to the number one complaint about SPR technology (that the surface will automatically change the thermodynamics of the system), what Dr. Pessimist fails to realize is that most biosensor experiments do not use a flat surface. Instead, the surface is coated with a dextran layer, which suspends the molecule in solution. We and others have shown with numerous systems that when experiments are performed properly, binding constants (including thermodynamic parameters) measured with SPR do in fact match those obtained from solution-based measurements.

However, I agree with Dr. Pessimist in one regard. Since 1991, I have read every paper that reported using a commercial SPR biosensor and Dr. Rebecca Rich and I have composed a yearly review of the literature since 1998. This is becoming a fairly daunting task since more than 1000 research papers are published annually. More, unfortunately, is not always better. We find that the data in most biosensor articles are not worth the paper they are printed on. For example, about half the time authors even fail to present figures showing the binding responses and yet they expect us to believe the rate constants they report for their interactions. Without a visual inspection of the data, we have no idea if the experiments were run properly. And oftentimes, even when data are presented, it is clear that the investigators do not know how to utilize the technology properly. Also, while a fundamental dogma of science is to replicate and randomize samples, less than 3% of published biosensor data include replicate injections even within a single experiment. An overlay of replicate injections demonstrates the stability of the reagents and multiple independent experiments yield an average and standard deviation for the reported binding constants, yet this attention to detail in a biosensor experiment is more rare than finding a four-leaf clover in the outfield at Fenway Park. In addition, less than 5% of the authors who report kinetic constants include an overlay of the binding response with the fitted model. And finally, even from a brief glance through the literature, it is apparent that the majority of investigators do not understand that the shape of the response profile should be an exponential in both the association and dissociation phases (maybe many users do not even understand what an exponential is). It is no wonder that scientists outside the biosensor use community think SPR technology does not work. I would think the same thing if all I had to rely on was the published data.

You might ask yourself, “how did it get to this point?” I often wonder if all scientists are so poorly educated in basic scientific technique (which could actually explain why we haven’t found a cure for the common cold). I place the blame on the “kit mentality” that was introduced with molecular biology back in the early 1990s, back when we were listening to our Walkmans while typing on our IBM 286 personal computers. Nowadays you can buy a kit to clone, mutate, express and purify a protein. Well, the kit mentality continued when these same investigators got access to commercially available biosensor technology. Since these instruments are so easy to use, anyone can walk up to the machine, chuck in their proteins, collect some response, fit the data and publish the results, believing that the results must be correct because they came out of this very expensive machine. Unfortunately, it actually takes some skill and know-how to set up, execute and analyze a biosensor experiment properly.

This leads me to the next group of biosensor users that give the technology a black eye. These people are the ones who have been using instruments for a long time and think they are experts. I call them “SPiRts”. SPiRts are even more threatening than Pessimists because their complacency often leads them to perpetuate poor experimental technique. A common SPiRt mistake published in the literature is the use of multivalent analytes in solution (*e.g.* monoclonal antibodies or GST fusion proteins), which can produce avidity effects. All too often, SPiRts present elaborate biological justifications for the shape of their unusual binding profiles when in fact the responses are simply indicative of poor reagent quality and/or inadequate experimental optimization or data processing. Even worse, SPiRts use complex models to describe their poor-quality data. It seems that the latest fad of these model surfers is to apply a conformational change mechanism. “My data fit a conformational change model, which must mean there is a conformational change, right?” Wrong!

To set the record straight, in 1994 my colleague and software engineer extraordinaire, Tom Morton (who I refer to as SoftEE), developed the numerical integration approach to data analysis that allows one to apply any interaction model. Before then, we were in the caveman days of linear transformation and, believe me, you don’t want to go back there. We were the first to show that a change in conformation that stabilized a bound complex would in fact produce a change in response even though there was no additional change in mass. However, in the intervening 13 years I have never needed to apply this model to describe the responses obtained from more than 1000 systems I have examined. The reason I am reluctant to use this model is that typically a data set that fits a conformational change model can be equally well described by other models such as those for ligand and/or analyte heterogeneity. Even more alarmingly, the rates for the supposed conformational changes measured on the biosensor are extremely slow, often with half-lives of 20–60 minutes if you take the time to calculate them. These rates do not make biological sense to me. A quick search of the classical conformational change literature shows that re-organizational events which occur during binding happen on a nanosecond to millisecond time-scale. The hot “new” trend with the SPiRts is to fit their biosensor data with a conformational change model

and then present crystal structure data of unbound and bound complexes and say "See, this change in conformation proves it". But an objective viewer would disagree. The fact that you see a change in conformation in the structure actually may not relate to the complex binding response you are measuring on the biosensor. Don't be fooled by these sleight-of-hand arguments. (What would help confirm the conformational change suggested by SPR would actually be to use a time-resolved structural method such as circular dichroism or fluorescence resonance energy transfer and demonstrate that the time-dependent changes are the same.) The cause of the complex binding response on the biosensor is actually more likely due to surface aggregation, nonspecific binding, molecular crowding, avidity effects or sample heterogeneity.

This brings me to my favorite SPR users, who I refer to as SPiRits. SPiRits are new users or those having some familiarity of biosensor technology who have a deep desire to learn more about its features, applications and potential. They are the ones who are participating in our yearly benchmark studies, which are geared toward calibrating users' experimental technique. They are willing to put in the effort to troubleshoot their systems and want to improve the quality of the data and not just settle for whatever the machine spits out. SPiRits will be the users who develop novel applications and implement new technologies in the future.

We need SPiRits because the number and types of SPR instruments are exploding. An Internet search reveals more than 20 companies developing SPR-based biosensor systems. Lately, biosensor advances have occurred on two fronts. First, many of the recently released instruments (and others currently under development) are dedicated to specific applications ranging from small-molecule drug discovery to the characterization of complex mixtures in the clinical and food sciences. Corning's Epic plate-based system is an example of targeting the technology for screening applications. Second, we have seen a push to increase the throughput of biosensor analyses. In the past few years, the launches of BioRad's ProteOn XPR36 and Biacore's A100 have dramatically impacted the biosensor field since they allow for parallel processing of multiple analytes over multiple targets simultaneously. Array-based platforms represent the next wave in biosensor development. Biacore's Flexchip and instruments being developed by GWC Technologies, Lumera, IBIS Technologies, Genoptics and Maven open up the possibility of characterizing hundreds to thousands of interactions at one time. But not surprisingly, these array formats come with their own sets of challenges. The methods used for spotting DNA may not be optimal for producing protein arrays. Clearly, a lot of work remains to be done before protein array systems meet their full potential.

As biosensor applications expand and new instruments are released, the technology's user base also increases. I worry that higher-throughput systems may allow more users simply to generate more bad data faster. So, we clearly need to improve the skill level of both novice and seasoned users.

This book is a great resource to obtain the fundamental knowledge of biosensor technology, and also discover recent developments in both

instrumentation and applications. But in order to turn professional, remember that the biosensor is just a tool. Use it wisely. Be skeptical, but keep an open mind. Know when to say when (not all systems are amenable to biosensor analysis). Go forth and become a good ShePaRd of my favorite technology.

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