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Survey of the 2009 commercial optical biosensor literature

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We took a different approach to reviewing the commercial biosensor literature this year by inviting 22 biosensor users to serve as a review committee. They set the criteria for what to expect in a publication and ultimately decided to use a pass/fail system for selecting which papers to include in this year's reference list. Of the 1514 publications in 2009 that reported using commercially available optical biosensor technology, only 20% passed their cutoff. The most common criticism the reviewers had with the literature was that "the biosensor experiments could have been done better." They selected 10 papers to highlight good experimental technique, data presentation, and unique applications of the technology. This communal review process was educational for everyone involved and one we will not soon forget. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: affinity; Biacore; biolayer interferometry; biomolecular interaction analysis; evanescent wave; kinetics; resonant mirror; surface plasmon resonance

When it comes to reviewing the commercial biosensor literature, we sometimes get the feeling that we are the only ones yelling "fire" in a crowded movie house that is actually on fire. If you are new to our literature reviews, we suggest you read over some of the past publications in this series (search "commercial optical biosensor literature" in *Journal of Molecular Recognition* between 1999 and 2009). In these reviews, we discussed the evolution of the technology and provided examples of how to (and not to) publish biosensor data.

Those of you who are familiar with our reviews know that for the past few years we have been taking a fairly critical look at the literature. In fact, last year, we went so far as to grade every paper on a scale of A to F.[1] Our goal was to educate readers about what to expect in publications and to help biosensor users improve their own work. We received some interesting responses to our last review, ranging from love to hate and one person even wrote a song about us. We will not bore you with the details, but the responses tell us that some of you are as passionate about the subject as we are.

We know this technology has a lot to offer, but frankly, we can tell from the literature that it is not being used to its full potential. Of course, sometimes we wonder if we should bother worrying about the quality of biosensor literature. Who cares that the average paper lacks sufficient detail to be replicated by another group or occasionally the reported binding constants are blatantly wrong? Perhaps we are hypersensitive to poor-quality data because we have been doing these reviews for more than 10 years now? Over this time, we have collected and read more than 10 000 papers that describe using biosensor technology. Maybe we need a break.

So we thought what better way to take a break than to take a Caribbean cruise—which we did—along with 22 other scientists as part of an advanced biosensor workshop in March of 2010.

These individuals are all biosensor professionals making a living applying the technology in different capacities. They work in industrial, government, and academic institutions. And combined, they have more than 150 years of experience using the technology. To put it into perspective, that is like one person running biosensor experiments since the invention of the light bulb. Some of these biosensor users work with antibodies and proteins, whereas others focus on small molecules and fragment screening. But they all share a common passion for biosensor technology, and because of kinetosis, each stared intently out the port side window as Cuba rolled up and down on the horizon. Gaining our equilibrium, we presented the first item on the meeting agenda: should we bother reviewing the biosensor literature? (So much for a break.)

ALL ABOARD!

En masse, the response was "yes." This group felt that critical reviews were essential to scientific progress. What the group did not know at the time was that they were about to become active participants in the review process. To avoid shipping costs and luggage handling fees, we had hauled more than 1500 papers from Salt Lake City to the Port at Miami. Apparently, transportation security has an extra pat-down procedure for travelers who have 100 pounds of paper in their carry-on luggage and no toothpaste.

The participants were divided up into pairs, each of which were given more than 100 papers to review. As a team, the pairs evaluated each paper in their packet to identify strengths and

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weaknesses in the literature while, we hoped, making sure their partner did not fall over board.

At first, we did not know how the participants would react to their task of reviewing the literature after full days of regular workshop presentations. Beyond the motion sickness, their reviewing assignment had to compete with the nightly Calypso Band, Legends Entertainment, and a host of fine dining establishments. And we were sure that no one would take their packet ashore when we docked at Grand Cayman and Cozumel. We were wrong. This group went to great lengths to be involved in the review process. As evidence, see Figure 1, which depicts four of the participants with papers in hand and palm trees in the background.

FULL SPEED AHEAD

Lesson no. 1 the participants learned from the review process was that when you read a big stack of papers all at one time, you get a better sense of how often critical information is missing in a publication. So the first thing we had to decide on as a group was what information should be required for a publication. The group came to the consensus that scientific reports should include enough information so that a reasonably skilled user could replicate the study. (Yeah, we know this is part of the scientific method, but we did not want to burst their bubble.) So they established a checklist of basic information (listed in the shaded box) that should be required to publish biosensor data.

Now interestingly, after we returned home from the meeting, one of the reviewers sent us an email of a timely paper by Bourbeillon *et al.* (*Nat Biotechnol* 28:650). This group is working on creating standards for protein affinity reagents and recently launched the Minimum Information About A Protein Affinity Reagent (MIAPAR) program:

> "[MIAPAR represents] an important first step in formalizing standards in reporting the production and properties of protein binding reagents…It defines a checklist of required information [that] would enable the user or reader to make a fully informed evaluation of the validity of conclusions drawn using this reagent…Although it is difficult to see how this could be anything other than a voluntary agreement, we hope that once this commitment is made by a critical mass of manufacturers, both commercial and nonprofit, it will become standard practice."

We were encouraged to see other groups attempting to bring some standardizing to their field. As a nod to the MIAPAR program, we named our checklist TBMRFAADOBE (The Bare Minimum Requirements For An Article Describing Optical Biosensor Experiments).

TBMRFAADOBE

- instrument used in analysis
- identity, source, molecular weight of ligand and analyte
- surface type
- immobilization condition
- ligand density
- experimental buffers
- experimental temperatures
- analyte concentrations
- regeneration condition
- figure of binding responses with fit
- overlay of replicate analyses
- model used to fit the data
- binding constants with standard errors

Figure 1. Scientists reviewing the optical biosensor literature on Grand Cayman Island, March 2010. Foreground to background: Olan Dolezal, David Stepp, Lucy Sullivan, and Jeff Dantzler.

WALK THE PLANK

In last year's survey, we graded each paper on a scale from A to F because our goal was to help authors recognize the quality of their biosensor experiments. But this review panel viewed the literature differently. They saw it more as a tool to help them in their own research. This makes sense when you think about it; that is what the primary purpose of publishing data should be. Make a breakthrough, discover something, publish it. Then others can learn and take it further. But what happens if what is published is incomplete or entirely wrong?

After much discussion, the group decided to use a pass/fail system with the overriding standards for a passing grade being "1. Would you rely on these data for your own work? 2. Would you recommend this paper to a colleague?" In some ways, this made the review process straightforward. They found it easy to select the outstanding papers and reject the awful ones. It was the papers in the middle that kept the reviewers busy debating until the wee hours every night and, in fact, made it an even better exercise for everyone.

Also, the reviewers decided that if no figures of binding responses were shown, they could not judge the reliability of the authors' conclusions and therefore would never use these papers in their own work. Therefore, 25% of the publications failed immediately because they did not include figures of the data.

In the end, the group developed specific criteria for a passing paper. They looked for lots of experimental details and evaluated the figures of data. Of course, what they wanted to see datawise depended on the assay (e.g., qualitative versus equilibrium or kinetic analyses), but all acceptable papers included plausible binding profiles with appropriate response intensities. The group also wanted to pass only those papers that showed overlaid responses from replicate analyte tests. But we had to eliminate this as a criterion because fewer than 20 papers of the 1514 included any replicates. It boggles the mind as to why so few scientists apply basic scientific principles to their own work. After all, the reason Columbus gets so much credit for discovering the New World was not because he sailed there once and never returned. Rather, he replicated his journey four times! Christopher would have made a great experimentalist.

The reviewers insisted the limbo bar for passing papers be even more stringent if the paper included binding constants. These papers needed to show at least one data set and fits to the data. Over the years, we have seen too many examples where the reported rate/affinity constants in no way describe the binding data (for specific examples, see our 2008 survey). And they insisted all binding constants should include the appropriate experimental errors, just like science papers in the movies. Most authors still have a lot of trouble with this.

For equilibrium analyses, the reviewers wanted to see responses of an analyte concentration series and an accompanying binding isotherm (no Scatchard plots; we all have computers now capable of nonlinear regression analysis. Thank you very much.). A paper automatically failed if non–steady-state data were fit using equilibrium analysis. Let us review. In an equilibrium experiment, all responses in a concentration series that you are going to use for analysis must plateau by the end of the analyte injection (wait, wait for it). Plateau means to be flat, to be parallel to the *X*-axis. Not still increasing—that would by definition not be "steady" state. The reviewers were shocked to see the number of researchers who erroneously apply an equilibrium analysis to non-equilibrium data.

For kinetic analyses, reviewers wanted to see the binding responses overlaid with a global fit of the interaction model. Also, there were a few things that got kinetic papers an automatic fail: model surfing (fitting data to several models; most often using a conformational change model because, as a number of authors claimed, "it fit the data best") or using the bulk–shift correction incorrectly. If you do not know what we mean by "using the bulk–shift correction incorrectly," then there is a good chance you are using it incorrectly. Please stop.

MAN OVERBOARD

According to the reviewers' reviewing criteria, of the 1514 papers published in 2009 that described biosensor analyses, only 20% received a passing grade. That means four out of five biosensor papers were deemed unacceptable. So it is no wonder that biosensor technology sometimes gets a bad reputation. Across the scientific literature and at conferences, we will occasionally see and hear remarks like "Results from the biosensor experiments do not agree with our other findings" and "We could not reproduce the biosensor results published earlier." Odds are, we now know why.

Several committee members commented that this becomes a significant issue when they are trying to manage expectations of their colleagues (who are not biosensor experts) with respect to the feasibility of reproducing reported results. We had reviewers tell us that when colleagues insisted that experiments be done according to some previous publication, they would reply "Okay, we could replicate those conditions and produce similarly incorrect results if you would like." In a way, this is a testament to the reproducibility of the technology. And many of the reviewers commented that oftentimes they do not even bother looking at the legacy biosensor data; as one person put it, "It's the last place we would start."

With this focused mindset, it is not surprising that the reviewers made the same comments over and over about their grading decisions when reviewing the 2009 literature. For failing papers, they frequently noted that the responses were "weirdly shaped." On the review sheets attached to each paper, they wrote comments like "These responses could not possibly describe a real binding event." "Clean the instrument!" "Why are these data not double referenced?" "These data are full of artifacts." "The interaction just looks complex because the surface density was way too high." "My eyes are burning, these data are so bad." Overall, the reviewers were stunned by how little attention researchers would pay to proper assay design and execution. The most common comment we saw on the review sheets was "This experiment might actually work but it needed a lot more optimization for these data to be believable." Bingo! (Binding Interactions Need Greater Optimization). This is the fundamental problem we see in the literature and in the real world. Many biosensor users take whatever comes out of the machine as gospel. Never questioning it, not really understanding it, and never trying to improve it.

Aside from the murmurs of an impending mutiny, we were actually happy to see the reviewers' frustrations with the literature. We were beginning to wonder if we were expecting too much from the research community.

CASTAWAY

Now as we were preparing this survey, another reviewer sent us an interesting commentary by Bauerlein *et al.* published in the June 2010 issue of *The Chronicle of Higher Education* entitled "We must stop the avalanche of low-quality research". Boy, the title says it all. It is as if Bauerlein and his colleagues had been with us during our review; talking about the scientific literature in general now, they echoed what we have been saying about biosensor literature for years:

> "While brilliant and progressive research continues apace here and there, the amount of redundant, inconsequential, and outright poor research has swelled in recent decades, filling countless pages in journals and monographs… Questionable work finds its way more easily through the review process and enters into the domain of knowledge… More isn't better. At some point, quality gives way to quantity."

We found another recent literature survey, this one by Schneider Chafen *et al.* (*JAMA* 303:1848). They screened more than 12 000 citations related to food allergy and concluded "there is a voluminous literature related to food allergy, but high-quality studies are few."

At times we feel like we are drowning in a sea of poor-quality biosensor papers. So we are happy to see reviewers in other disciplines point out the shortcomings in their respective fields. As we have been arguing for years, there is nothing fundamentally wrong with biosensor technology. The reports by Bauerlein *et al.* and Schneider Chafen *et al.*, as well as our own reviews, suggest that a large number of scientists may be ignorant, arrogant, or lazy. But at least knowing that we are not alone as we struggle to improve our own field is comforting.

THROW ME A LIFE PRESERVER

Of course not all is doom and gloom with the biosensor literature. The number of good-quality papers is increasing slowly, and we would like to believe that we may have contributed to this improvement by hammering on the basics over the past few years. In the 316 passing papers, the reviewers found a number of particularly high-quality data sets from kinetic, equilibrium, concentration, and qualitative analyses. They were delighted to see the effort some authors put into their assay design, experiment execution, and data presentation. Passing papers got comments like "That's what a binding response should look like." "Nice simple-exponential curves." "These authors were smart enough to prepare low-density surfaces." "Hey, I see replicates. Look everyone, gather round. Here are replicates!" and "Nice example of showing similar binding constants were obtained by testing the system in both orientations." At the end of the meeting, the group gathered to discuss and vote on each of the top papers selected by the review teams. It was a bit like being at the Colosseum in ancient Rome, only with 22 Caesars.

Full references for the 316 papers that were given the thumbs up are provided in the reference section. To make this list useful as a tool for identifying examples of the various assay formats, the citations are subgrouped by reviews/protocols dedicated to biosensor studies,[2–39] kinetic,[40–150] equilibrium/competition,[151–186] or concentration analyses,[187–199] qualitative formats (e.g., yes/no, screening, epitope mapping),[200–291] and novel surface preparations.[292–317]

HIGH TIDE

The reviewers also chose 10 primary research articles (listed in the gray box) that deserved special recognition for their use of biosensor technology. These papers demonstrate the impact and versatility of biosensor technology in diverse research programs. In addition, they all describe well-performed experiments and include a wealth of figures of binding data. Universally, the figures show reasonable response levels for analyte binding (indicating the surface densities were not too high or too low), and the responses are reasonably shaped. Shape is everything for biosensor data. Knowing how it should look, and what to do when the shape is not right, distinguishes the biosensor professional from the novice.

For each highlighted paper, we summarized how the biosensor studies contributed to the project, show examples of data, and describe some of the outstanding technical features of these experiments. These 10 papers represent the high water mark of the 2009 commercial biosensor literature.

Assay Development

Indyk *Int Dairy J* 19:36
Abdiche *et al. Anal Biochem* 386:172

Novel Immobilization

Hosse *et al. Anal Biochem* 385:357

Well-Performed Experiments

Harris *et al. J Biol Chem* 284:9361
Huang *et al. J Mol Biol* 392:1221
Magotti *et al. J Mol Recognit* 22:495
Persson *et al. J Virol* 83:673
Pope *et al. J Immunol Meth* 341:86

Intriguing Application

Hayashi *et al. Chemistry* 15:424

User Evaluation/Technology Validation

Rich *et al. Anal Biochem* 386:194

Assay Development

Indyk. Analyte detection and quantitation. *Int Dairy J* 19:36.

Although optical biosensors are well established as a detection/quantitation tool in the food, veterinary, clinical, and environmental testing industries, we rarely see papers that detail the parameters required to develop this assay for a new analyte and/or include figures of binding data. Indyk's report does both exceptionally well, illustrating the various steps of assay design/validation for testing α-lactalbumin in milk and its derivatives[189]. He screened commercial α-lactalbumin binders to identify a suitable testing agent (Figure 2A); determined the sensitivity, throughput, and sample consumption of several assay formats (Figure 2B-D); and optimized other conditions (e.g., ligand selection, immobilization chemistry, regeneration solutions, analyte concentration/contact time) to obtain reproducible binding (Figure 2E). As additional validation steps, Indyk established that this biosensor-based approach correlates well with alternative analytical methods across a wide detection range (Figure 2F) and could track the decrease in α-lactalbumin content in milk produced in the first days of lactation (Figure 2G). These methods are readily adaptable to developing assays to detect/quantitate analytes in a variety of crude samples and Indyk's work serves as the archetype for reporting this type of analysis.

Abdiche *et al.* Epitope binning. *Anal Biochem* 386:172.

Recognizing that determining the binding region within an antigen rather that the binding affinity is most often the critical first step in developing a therapeutic antibody, Abdiche *et al.* established three epitope binning assays and evaluated each using instruments manufactured by Biacore, BioRad, and ForteBio[200]. This group described important factors to consider when choosing when to use each assay, discussed the advantages and throughput of each instrument, and demonstrated that similar epitope bins were identified by the different assay formats and instruments.

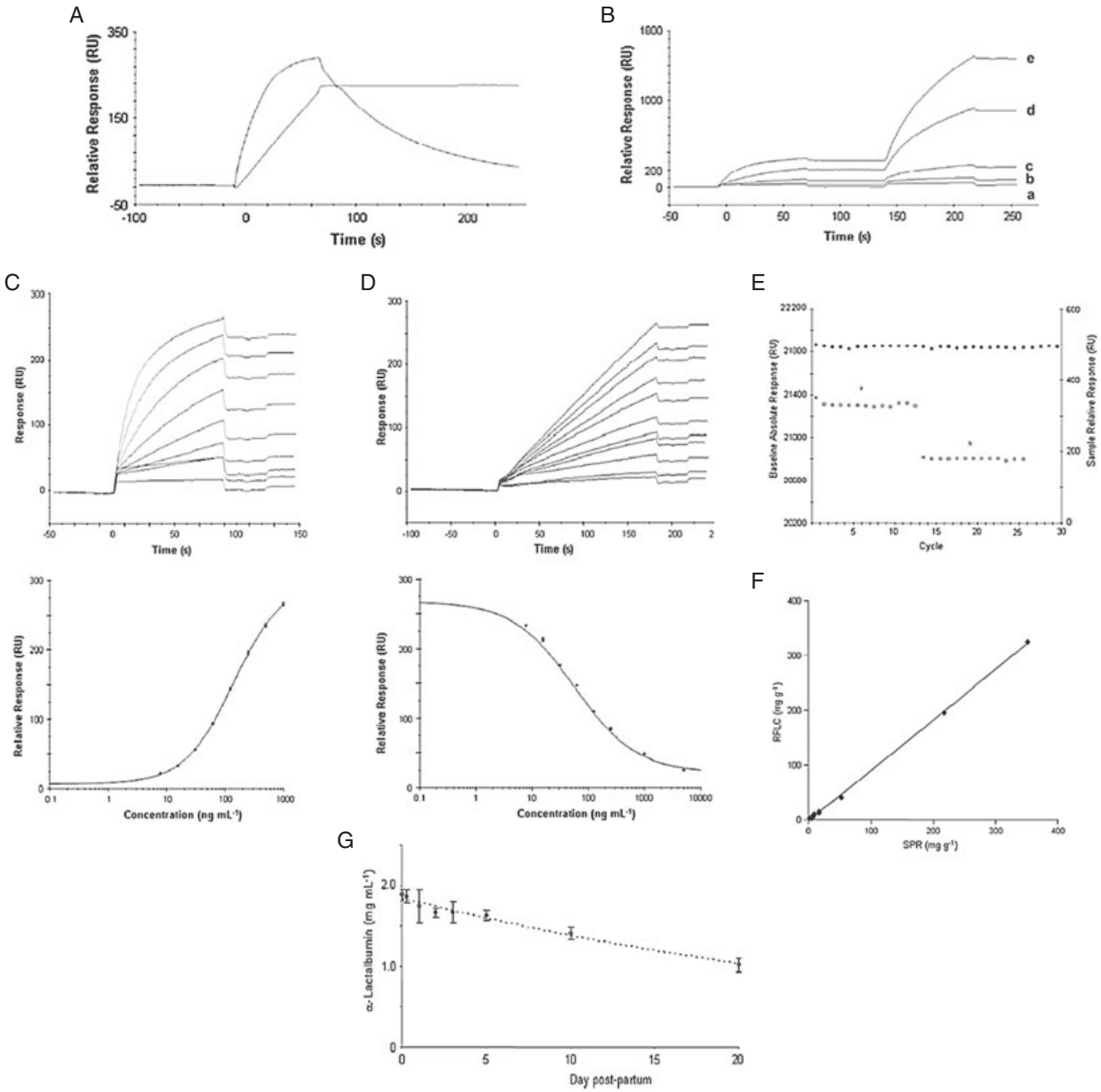

Figure 2. Development of an α-lactalbumin immunoassay. (A) Screen of two α-lactalbumin binders. (B) Enhanced binding approach: injections of five α-lactalbumin concentrations (a–e) followed by injections of anti–α-lactalbumin across an α-lactalbumin surface. (C) Responses and isotherm obtained from the direct binding assay. (D) Responses and dose-dependent curve from the inhibition assay. (E) Reproducibility of replicate tests of (a) α-lactalbumin standard and (b) whey protein isolate. The responses from the baseline are included as a reference (filled squares). (F) Correlation between the α-lactalbumin detected by biosensor and reverse-phase liquid chromatography. (G) Biosensor-determined bovine α-lactalbumin content in early lactation milk. Reprinted from reference 189 with permission from Elsevier © 2009.

Abdiche *et al.* provided extensive experimental details for the three assay designs and outlined how each was adapted for the individual biosensor platforms. These authors also included a wealth of figures that clearly illustrated the responses obtained for competing and non-competing antibodies, as well as the different signals obtained from the three instruments (Figure 3).

Novel Immobilization

Hosse *et al.* Novel ligand capture and rigorous kinetic analyses. *Anal Biochem* 385:357.

Hosse *et al.* established a new capturing system based on the high-affinity interaction of *Escherichia coli* colicin E7

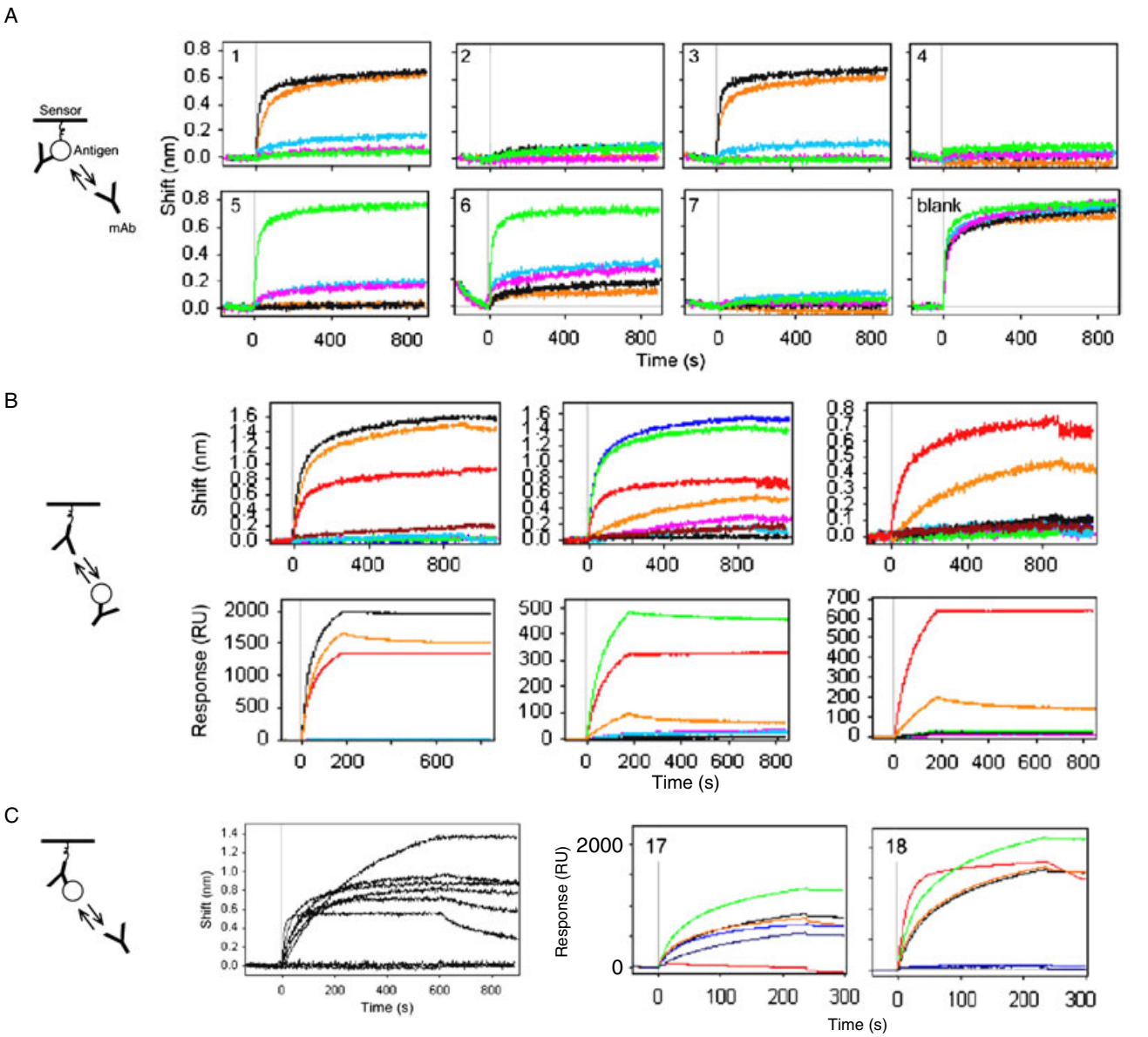

Figure 3. Epitope binning with three assay formats. (A) In tandem blocking of competing mAbs in solution screened against pre-formed Ag/mAb complexes measured using Octet QK. (B) Premix blocking of Ag/mAb complexes in solution screened against three immobilized mAbs (left to right) measured using Octet QK (top) and ProteOnXP36 (bottom). (C) Responses from a classical sandwich format for mAbs screened using the Octet OK (left) and ProteOnXPR36 (right). Reprinted from reference 200 with permission from Elsevier © 2009.

(DNaseE7) and immunity protein 7 (Im7)[67]. Im7-tagged ligands are readily captured by immobilized DNaseE7, the captures are stable and reproducible, and the DNaseE7 surfaces are easily regenerated and active for a long time (Figure 4A). In addition, tagged ligands can be efficiently captured from crude supernatants (Figure 4B), and the tag does not affect the inherent kinetics of the ligand/analyte interaction. Even alone, a report of this new capture system would have been impressive. But that was not all; these authors also described exceptional kinetic analyses for four different biological systems (Figures 4C–4F.). From a biosensor user's viewpoint, these analyses were outstanding for several reasons. Using low ligand capture levels and an appropriate range of analyte concentrations, Hosse *et al.* obtained reliable responses that were well described by a simple interaction model for each of the four systems. In addition, the analyses were so reproducible that is it difficult to distinguish between the replicate responses included in Figure 4C–E.

Well-Performed Experiments

Harris *et al.* Excellent use of kinetic and equilibrium methods. *J Biol Chem* 284:9361.

The characterization of apical membrane antigen 1 (AMA1)-binding peptides done by Harris *et al.*[66] epitomizes what the

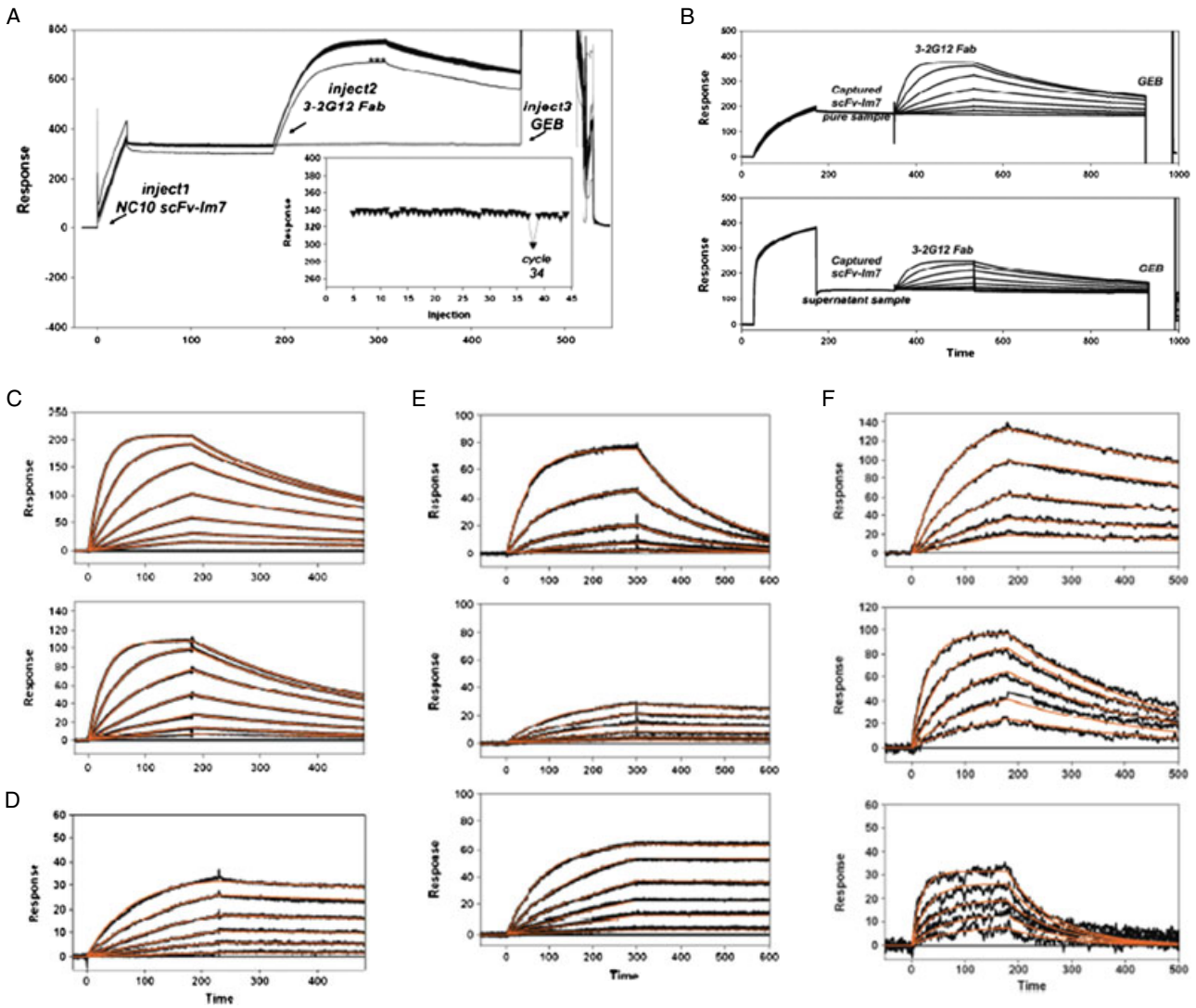

Figure 4. Capture and kinetic analyses of Im7 conjugates. (A) Reproducibility of NC10 scFv-Im7 capture and activity. (B) Overlay of full binding cycles of a 3-2G12 Fab concentration series binding to purified and crude preparations of NC10 scFv-Im7. (C) Responses for 3-2G12 binding to captured NC10 scFv-Im7 (affinity-purified, top panel; crude, bottom) fit to a 1:1 interaction model (red lines). (D) Carcinoembryonic antigen binding to captured T84.66 scFv2-Im7 diabody. (E) Ricin binding to three anti-ricin Fab-Im7s captured from culture supernatants. (F) Apical membrane antigen 1 binding to three captured 12Y-2 V_{NAR}-Im7 clones. Data in panels A-E were collected using Biacore T100; data in panel F were collected using BioRad ProteOn XPR36. Reprinted from reference 67 with permission from Elsevier © 2009.

reviewers wanted to see in papers describing biosensor-based kinetic and equilibrium experiments. Not only are the data in each panel in Figure 5 easy to see but also the panels include replicates; the reproducibility of the responses from triplicate injections of each peptide concentration established the reliability of the analysis. In addition, the signal intensities are fairly low to minimize potential complexity in these interactions. Particularly notable is the appropriate use of kinetic and equilibrium fitting methods in the study by Harris *et al.* The data sets in Figure 5A were fit to a kinetic model because there was enough curvature in the dissociation phase to define the rate constants. In contrast, the responses of every concentration in Figure 5B have plateaud by the end of the sample injection, so an equilibrium analysis can be applied to the entire concentration series.

Huang *et al.* Thorough analysis and presentation of data. *J Mol Biol* 392:1221.

This snapshot in Figure 6 of Huang *et al.*'s protein engineering work exemplifies how well-performed biosensor experiments help direct a research program. By monitoring how manipulating the domain interface of a synthetic protein affected the binding constants, this group worked toward identifying critical contacts in the peptide/protein interface and increasing the interaction specificity[68]. In the matrix of interactions show in Figure 6, improvements in affinity are apparent across the sequential protein generations (from top to bottom), as are the effects of alanine substitutions within the peptide (right to left).

Experimentally, Huang *et al.* made several wise choices. The ligand densities they used produced relatively low analyte binding signals, which are double referenced and of the shapes

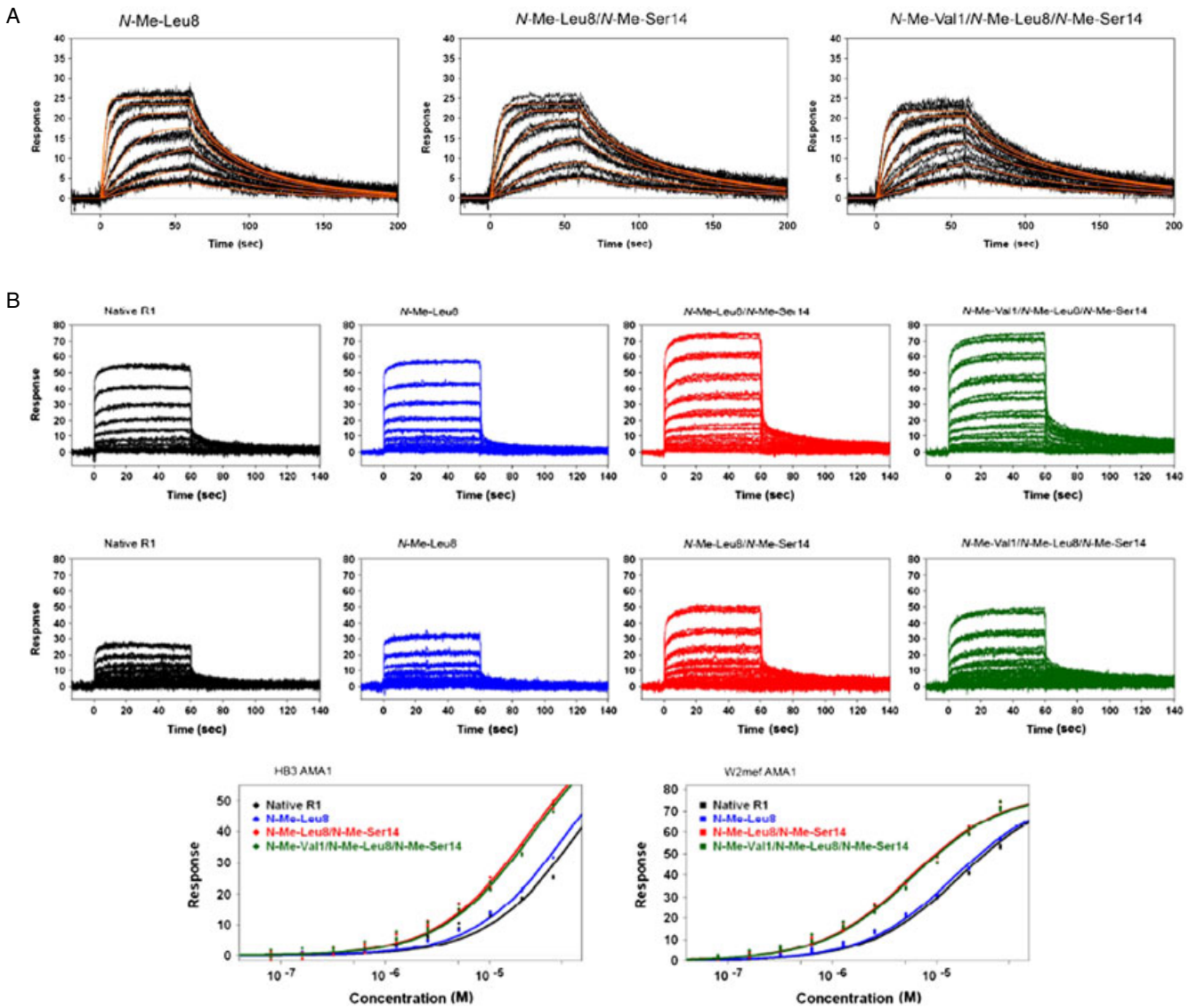

Figure 5. Kinetic and equilibrium analyses of native and mutated R1 peptides binding to immobilized AMA1. (A) The fit of a 1:1 interaction model is overlaid atop the responses collected for three mutant peptides/AMA1 interactions, with triplicate responses of each peptide concentration shown. (B) Triplicate responses (top) and isotherms (bottom) obtained for four weaker-affinity peptides binding to two forms of AMA1. Reprinted from reference 66 with permission from the American Society for Biochemistry and Molecular Biology © 2009.

we would expect for these binding events. In addition, for each interaction, the analyte concentrations were well chosen, producing responses ranging from near saturation to almost no binding. A wide analyte concentration range helps to test the reaction mechanism. These authors also included a lot of easily interpretable data sets. Just by looking at Figure 6, a reader can immediately identify which mutations improved or disrupted the protein/peptide interaction, as well as judge how well the data are described by the fitted rate constants. And, it is nice to see figures plotted so that the data and labels are legible.

Magotti *et al.* Kinetic analyses and corroborating experiments. *J Mol Recognit* 22:495.

Toward developing next-generation therapeutics against the complement system, Magotti *et al.* evaluated how hydrophobicity and backbone modifications in compstatin affected this peptide's binding to C3b[96]. Not only did they identify which substitutions enhanced the interaction, but the kinetic studies also revealed modification-specific trends and effects that were not apparent from steady-state enzyme-linked immunosorbent assay (ELISA) and isothermal titration calorimetry (ITC) analyses. Furthermore, this work demonstrates the care required to obtain high-quality kinetic data from a biosensor experiment. Magotti *et al.* used the initial compstatin screening information (Figure 7A) to optimize the concentrations of each analog to be used in the detailed kinetic analyses shown in Figure 7B; each analog required a specific concentration range to achieve binding responses that ranged from near saturation to almost no binding. In addition, the complementary ELISA and ITC measurements confirmed that capturing C3b on the sensor surface did not alter the binding parameters (Figure 7C).

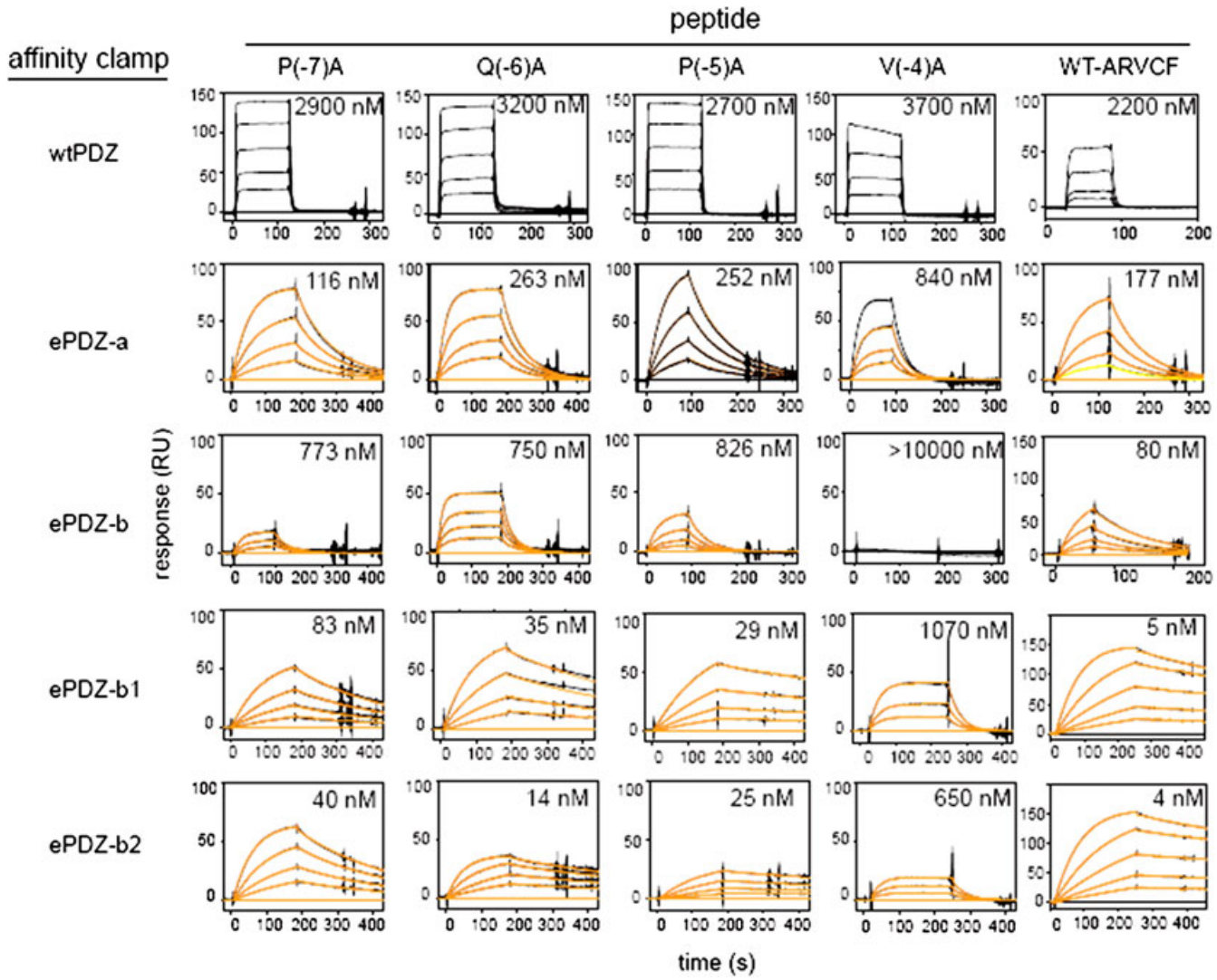

Figure 6. Responses and fits for Ala-substituted peptides binding to successive generations (from top to bottom) of engineered proteins. The fit of the 1:1 interaction model is overlaid atop each data set fit using kinetic analysis. Affinities of the weakest interactions (top row) were determined using equilibrium analysis. Reprinted from reference 68 with permission from Elsevier © 2009.

Persson *et al.* Direct kinetic and equilibrium vs. indirect competition analyses. *J Virol* 83:673.

Taking advantage of the biosensor's flexibility in assay design, Persson *et al.* developed complementary assays to characterize adenoviruses (Ads) binding to the CD46 cellular receptor (Figure 8)[112]. From direct kinetic and equilibrium analyses, these researchers identified a single residue in the Ad knob protein that is critical for high-affinity binding to CD46 (Figures 8A and 8B). Using a solution–competition analysis, they established that members of an Ad knob subfamily all bind at the same site in CD46 (Figure 8C). From a biosensor user's perspective, the data obtained from these experiments are outstanding for several reasons. The responses for the kinetic and equilibrium analyses are low, the duplicates overlay, and the data are well described by a simple interaction model. In addition, the multiple steps required in a rigorous solution competition analysis are illustrated in great detail and clearly described in the text.

Pope *et al.* Kinetic screening. *J Immunol Meth* 341:86.

Pope *et al.* designed a hybridoma supernatant screening assay that ranks antibodies by affinity, activity, and concentration[115]. Their approach allows for the determination of binding constants in higher throughput than is obtainable with standard kinetic assays. Although similar methods have been described previously for larger antigens, these authors outline the challenges encountered, as well as the optimizations used, to detect and characterize small peptides (<2 kDa) in solution binding to surface-tethered rabbit monoclonals (Figures 9A–9D). And, to confirm the validity of the parameters obtained from the kinetic screen, Pope *et al.* performed full kinetic analyses of several promising antibodies (Figure 9E). These methods (described in great detail by the authors) should be widely applicable for kinetically ranking targets that have relatively small binding partners.

Technically, this work is outstanding because it avoided the pitfalls that can be encountered in biosensor-based screening

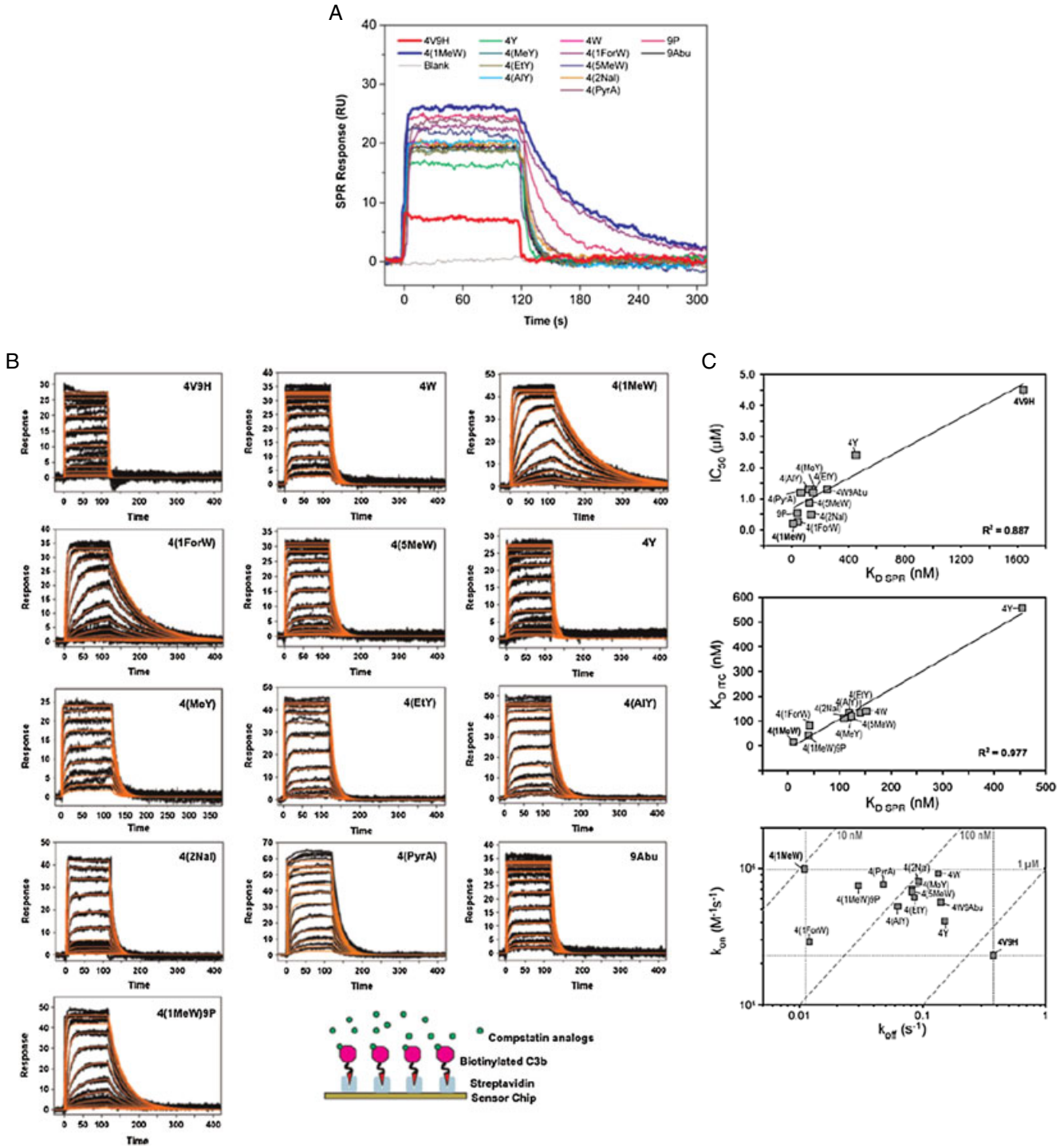

Figure 7. Structural modifications in compstatin that affect its binding to C3b. (A) Kinetic screening of compstatin analogs. Thirteen peptides were tested at the same concentration for binding to surface-tethered C3b. (B) Full kinetic analyses of the 13 analog/C3b interactions. Red lines depict the fit of the responses (duplicates at each compstatin concentration) to a 1:1 interaction model. A cartoon illustrating the assay design is included at the bottom of the panel. (C) Correlations between binding parameters. Top: activity (half maximal inhibitory concentration [IC_{50}], ELISA) versus affinity (K_D, SPR); middle: solution-determined affinity (K_D, ITC) versus surface-determined affinity (K_D, SPR); bottom: k_a versus k_d, with dashed isoaffinity lines included. K_D = equilibrium dissociation constant; K_a = association rate constant; K_d = dissociation rate constant. Reprinted from reference 96 with permission from John Wiley & Sons, Ltd. © 2009.

assays. For example, rather than immobilizing the peptide, Pope *et al.* captured the antibodies on the surface to avoid avidity effects that would produce artificially tight binding parameters. Also, they screened several anti-rabbit IgGs to find an optimal capturing agent (i.e., one that readily and stably bound their antibody but was also easy to regenerate).

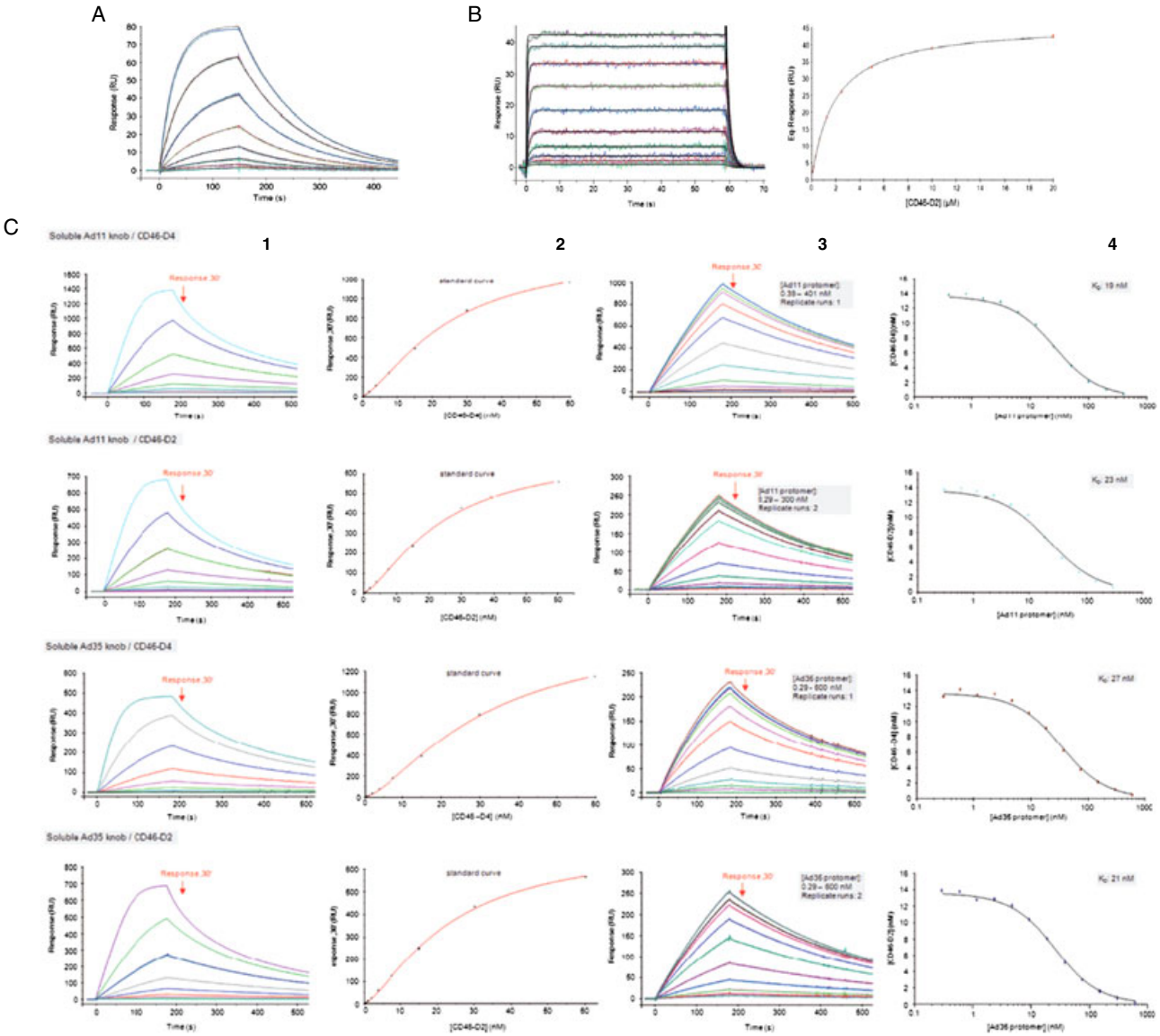

Figure 8. Analyses of CD46/Ad knob interactions. (A) Kinetic analysis of CD46 binding to immobilized Ad11 knob, with the fit of a 1:1 interaction model overlaid atop the duplicate responses obtained for each CD46 concentration. (B) Kinetic and equilibrium analyses of CD46 (tested in duplicate) binding to a mutant Ad11 knob surface. (C) The four steps (labeled 1–4 left to right) in solution–competition analyses of soluble Ad11 and Ad35 knobs competing with immobilized Ad35 for binding to CD46. Reprinted from reference 112 with permission from the American Society for Microbiology © 2009.

Intriguing Application

Hayashi *et al*. Innovative experimental design. *Chemistry* 15:424.

Hayashi *et al.* described using the biosensor to track reversible aptamer binding to a peptide that undergoes structural changes upon exposure to different wavelengths of light[229]. To monitor photoisomerization using Biacore 2000, the peptide-immobilized sensor chip was removed from the instrument, irradiated, and redocked in the instrument for analysis (Figure 10A and 10B). With Toyobo SPR-200, the aptamer/peptide complexes were monitored in real time by photoirradiating the surface through a window built into the instrument (Figure 10C).

We applaud Hayashi *et al.* for being bold but not foolhardy. These researchers went to great lengths to verify their atypical results. They tested several aptamers against the same peptide surfaces and obtained similar results using both traditional and imaging biosensor platforms. Even more importantly, they included suitable controls: both reference spots on the sensor surface and blank buffer injections.

User Evaluation/Technology Validation

Rich *et al.* Global benchmark study. *Anal Biochem* 386:194.

To establish how reported rate constants vary when users design their own experiments and use a variety of biosensor

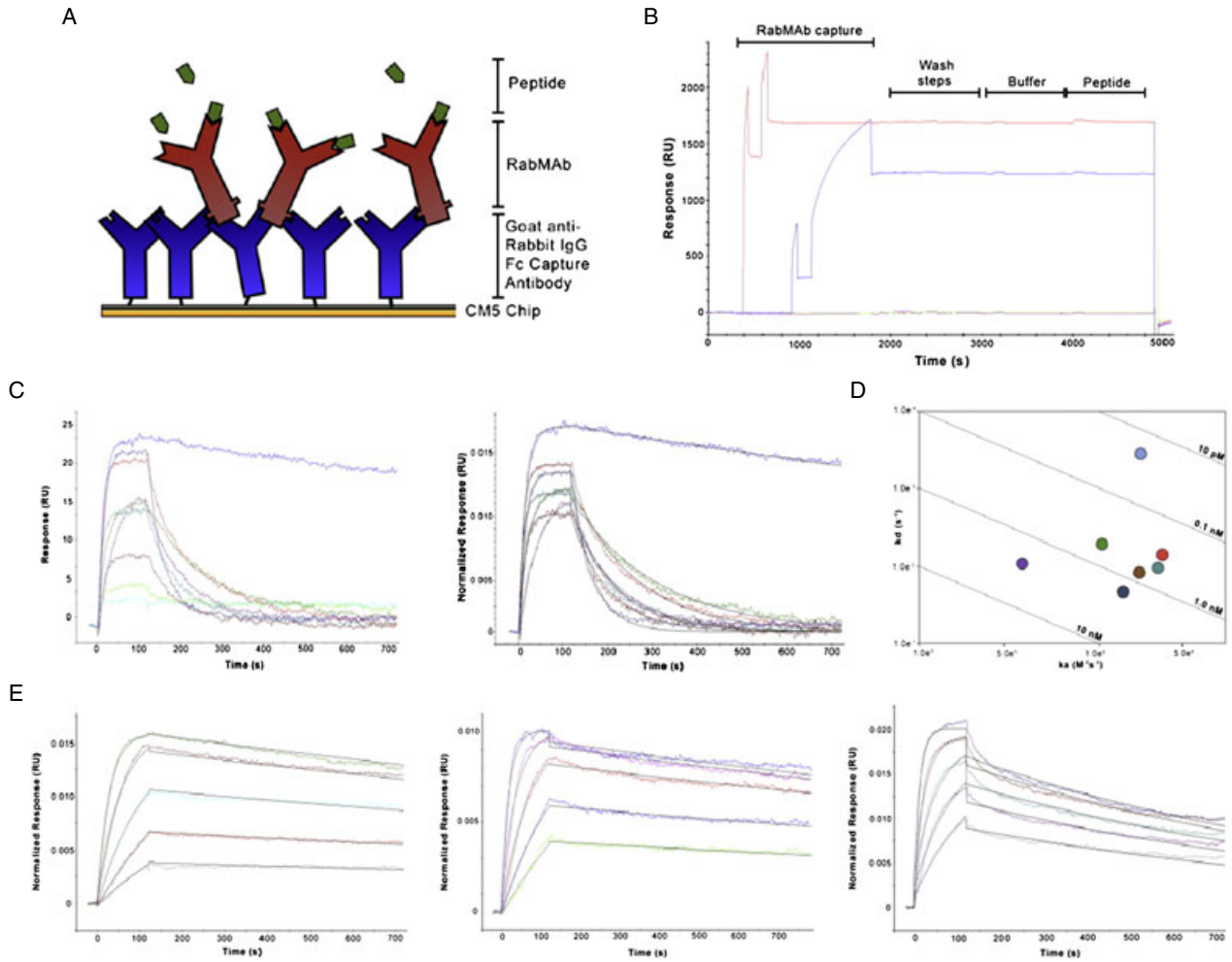

Figure 9. Ranking anti-peptide antibodies from hybridoma supernatants. (A) Assay design for the kinetic screening of anti-peptide RabMAbs. (B) Full binding cycle for the testing of two RabMAbs. (C) Overlay of representative peptide binding responses (left) and the fit of these responses (normalized for capture level) to a 1:1 interaction model (right). Responses of <5 resonance units in the left panel were omitted from the kinetic fitting. (D) Kinetic distribution plot of selected RabMAbs. Diagonal lines depict affinity isotherms. (E) Rigorous kinetic analyses of three RabMAb/peptide interactions. Reprinted from reference 115 with permission from Elsevier © 2009.

platforms, we provided aliquots of two binding partners (a 50-kDa Fab and a 60-kDa GST-tagged Ag) to 150 volunteers and asked them each to determine the kinetics of the interaction[122]. The participants were free to explore a wide range of experimental parameters. The top set of panels in Figure 11 show the data sets we received from 10 participants and the bottom panels in Figure 11summarize the results from all 150. Overall, the rate constants determined by the participants agreed well regardless of which binding partner was tethered to the surface and which instrument was used. Particularly informative were the data sets that were outliers. By examining these responses, it was apparent that the design and/or execution of many of these experiments could be optimized. Although this benchmark study demonstrated that reliable rate constants are obtainable by independent investigators, the ability of this group to generate high-quality data is not necessarily representative of the biosensor community. The participants in this study volunteered to run the analysis. The fact that they are willing to work on these types of benchmark projects means they are likely to have a higher interest in the technology.

BON VOYAGE

To give our vocal cords a rest this year, we enlisted the help of 22 individuals who make their livings from biosensor technology. Although the review process was discouraging at times, it certainly was enlightening to all of those involved. The general criticism of the publications was that the experiments could be done better. No one can argue that the low percentage of high-quality papers is associated only with biosensor technology because it appears to be pervasive throughout the scientific community. Now we could just throw our hands in the air and wave them like we just don't care, or we could work to put biosensor technology on a tack to have the highest percentage of high-quality data. After all, if something is worth doing isn't it worth doing a second time, only better?

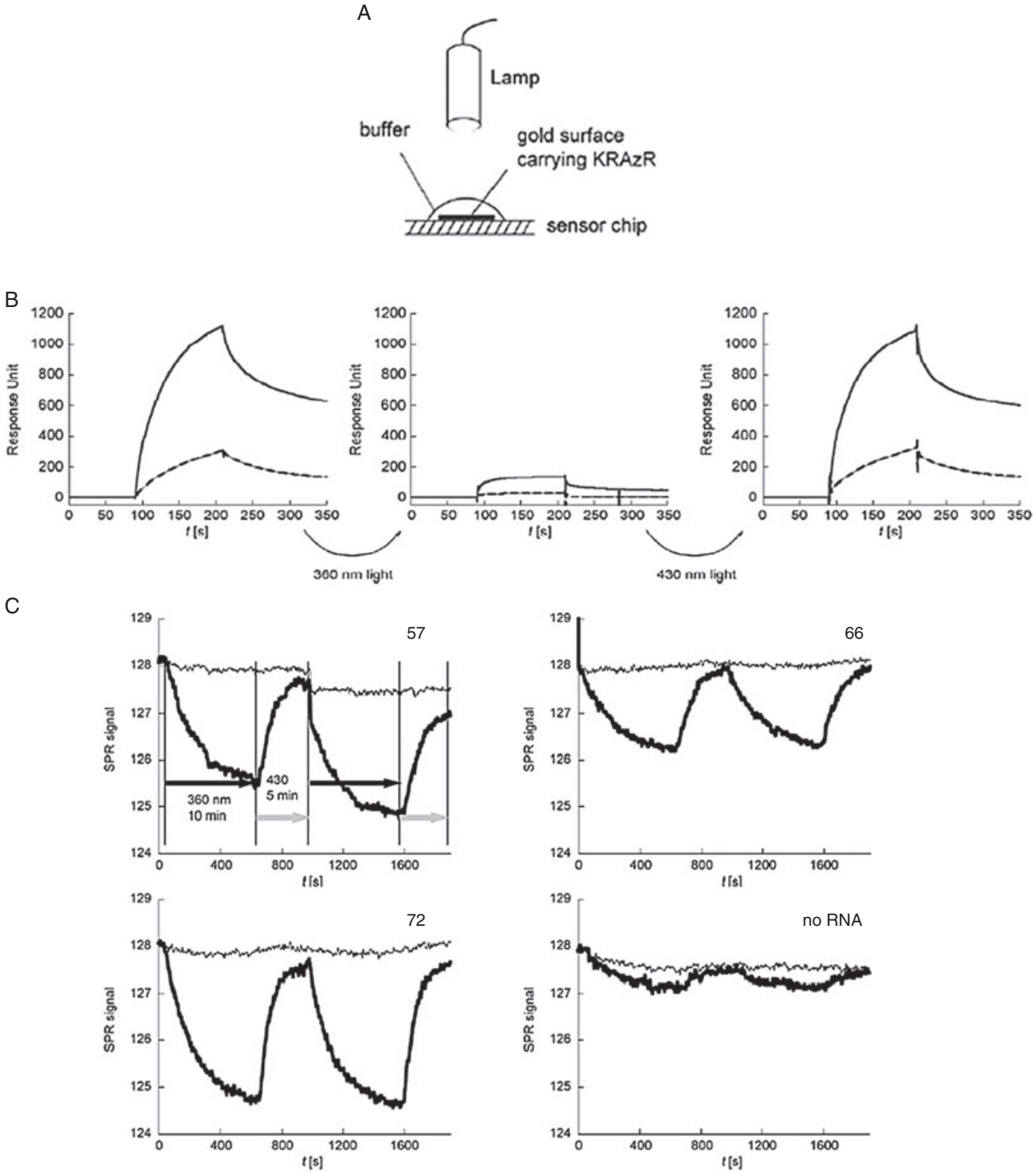

Figure 10. RNA binding to a photoresponsive peptide, KRAzR. (A) Off-line irradiation of peptide immobilized on a Biacore sensor chip. (B) Responses obtained using Biacore 2000 for two aptamers (indicated by the solid and dashed lines) injected across the peptide surface before (left) and after photoirradiation at 360 nm (middle) and 430 nm (right). (C) Real-time monitoring using Toyobo SPR-200 of the photoresponsiveness of three aptamers (designated 57, 66, and 72) binding to KRAzR. The bottom right panel shows the signals obtained for a buffer blank test. In each panel, the thin gray lines depict the signals from reference positions on the sensor surface. Reprinted from reference 229 with permission from Wiley-VCH Verlag GmbH & Co. © 2009.

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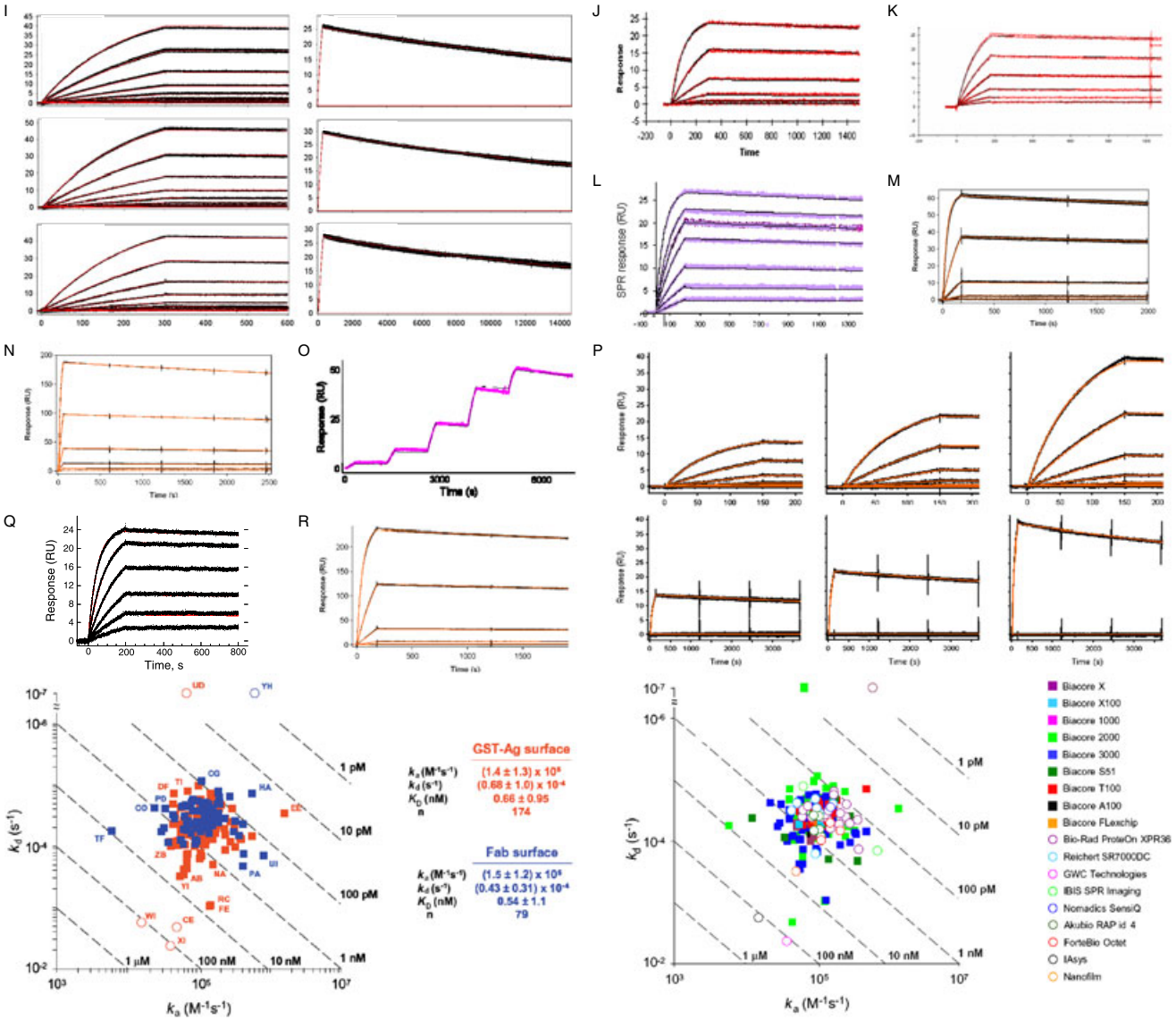

Figure 11. Global benchmark study. (top) Data sets collected by participants I through R. (bottom) k_d versus k_a plots of the kinetic parameters determined by the participants. (bottom left) Analyses of Ag surfaces are shown in red, and Fab surface are shown in blue. (bottom right) Analyses grouped by biosensor platform. Reprinted from reference 122 with permission from Elsevier © 2009.

(Wasatch Microfluidics), Jacek Nowakowski (Celgene), Ashique Rafique (Regeneron), Jason Simmonds (Cephalon Australia), David Stepp (Genzyme), Lucy Sullivan (University of Melbourne), Denise Wagner (International AIDS Vaccine Initiative), Charlotte Wiberg (NovoNordisk), and Mohammed Yousef (BioRad).

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