Monoclonal antibodies are key tools for basic research as well as for diagnosis and treatment of human diseases. Therapeutic monoclonal antibodies approached $50 billion in worldwide sales in 2010, and six of the current top twenty pharmaceuticals are monoclonal antibodies.

The expanding demand for high-quality antibodies with better specificities has resulted in a significant improvement in traditional hybridoma production methods, allowing production of a large number of antibodies against a target antigen. This has created an increasing demand for screening methods that characterize antibody-antigen interactions.

With the rapidly increasing pace of screening also comes the need to more fully characterize the antibodies at an earlier stage to improve the efficiency of the antibody selection process. The traditional approach of using end-point ELISA techniques to identify high-affinity antibodies provides limited information and suffers complications due to interference by tags. By contrast, label-free screening methods provide kinetic information on antibody-antigen interactions that lead to reliable estimation and ranking of antibodies in their affinity for the antigen.

ForteBio’s Octet platform provides a microfluidics-free, higher throughput, label-free kinetics platform for screening antibodies in complex samples against target antigen. By analyzing directly in crude media without costly and time-consuming purification steps, the Octet platform saves valuable time and effort.

With the recent launch of the Dip and Read™ Anti-Mouse IgG Fc Capture (AMC) biosensors, kinetic characterization of macromolecular interactions of mouse antibodies and other mouse Fc-containing proteins against target analytes is easier than ever. Cost-effective regeneration of the biosensors and the ability to directly immobilize mouse Fc-containing proteins from crude matrices make the AMC biosensor extremely useful in high-throughput applications.

AMC biosensors provide a flexible platform for evaluating the kinetics between mouse Fc-containing proteins and their analytes. AMC biosensors enable:
- Complete kinetic analysis ($k_a$, $k_d$, and $K_D$) between mouse Fc-containing proteins and target analytes
- Off-rate ranking of hybridoma and stable cell-line supernatants
- Epitope binning/mapping (from crude or purified samples)

continued on page 9
Over the last seven years, ForteBio and its Octet® platform have traveled an exciting journey with our customers. With their support, ForteBio, based in Menlo Park, CA, with subsidiaries in London and Shanghai, has become the second-leading—and fastest-growing—provider of label-free analysis tools for the pharmaceutical and biotechnology research fields, according to leading independent market analysis firms.

We launched our first product, the Octet QK system for label-free, real-time detection and analysis of protein-protein interactions, at the Antibody Engineering conference in San Diego in 2005. The revolutionary Octet platform accelerates protein and antibody discovery and development by generating real-time quantitative and kinetics results in higher-throughput formats, quickly and easily. Our ready-to-use, Dip and Read™ disposable biosensors enable researchers to measure concentrations and kinetic rates of critical samples, even in crude matrices, with unprecedented ease of use and cost-effectiveness.

Since then, customer response to our technology and products — including our broadening menu of 13 “off-the-shelf” biosensors — has been overwhelming. To date, we have shipped more than 4 million biosensors and, by 2010, more than 100 literature citations referenced our technology, signaling its strong adoption.

ForteBio’s web site hosts many end-user presentations from scientific conferences detailing productivity gains achieved via Octet. In addition to employing our off-the-shelf biosensor chemistries, users continually develop compelling new applications for our technology by immobilizing a ligand of interest to Streptavidin or Amine-reactive biosensors. Some of these applications include contaminant testing, vaccine development and virus-like particle analysis.

We are constantly working to improve and expand the Octet platform. In March 2008, we launched our second-generation system, Octet RED, whose extended sensitivity and reduced noise enabled small molecule drug discovery applications such as interaction analysis of fragment screening and peptides analysis. The third-generation Octet 384 systems further advanced the platform with automation capabilities and unattended operation in both 96- and 384-well plate formats. In 2010, biosensor re-racking was added to the product line, augmenting workflow flexibility and decreasing running costs.

The wide range of microfluidic-free Dip and Read™ biosensors is key to the Octet platform’s ease of use and fast time to results. For example, Protein A biosensors have facilitated antibody titer in cell line development with quantitation of 96- or 384-well sample plates in minutes, offering a strong alternative to ELISA and HPLC assays, which are labor-intensive and have a lag in time-to-result. Streptavidin biosensors allow researchers to immobilize custom molecules and conduct tailored quantitation, kinetic and affinity measurements.

ForteBio’s biosensor-based solution has two components: a low cost per data point, enabling customers to adopt the Octet platform broadly; and complete solution kits and methods, such as the Host Cell Protein and Residual Protein A assays which include biosensors and requisite reagents. The Octet system has also become a strong tool to complement or replace SPR (surface plasmon resonance) techniques, in which instrument running cost and throughput inefficiencies hinder research productivity. In fact, many Octet users tell us they routinely run antibody screening, epitope binning and affinity characterization studies — applications typically conducted with SPR — using the Octet system.

Our customers have also strongly adopted our recently launched biosensor chemistries and other enhancements including Protein G, Protein L and anti-murine biosensor chemistries, as well as new software tools in Octet Software version 7.0.

ForteBio continues to experience tremendous success, having achieved double-digit growth over the last few years. We could not have accomplished this without the ongoing partnership and support of our valued customers. We thank them and look forward to much continued success together in the coming years.

Christopher M. Silva, Vice President, Marketing, csilva@fortebio.com
NEW! Octet Software Version 7

Wesley Straub, Product Manager, wms@fortebio.com.

Octet software v7.0 for data acquisition and data analysis is now available for all Octet systems. Exciting new features in v7.0:

DATA ACQUISITION

Replicate Groups
Organize replicate samples into groups for convenient calculation of average concentration, average binding rate, standard deviation and CV%. Statics are automatically calculated during data analysis and can be plotted in the new X-Y graphing tool for custom viewing.

Grouped View for Quantitation
Simplify the visual analysis of quantitation datasets with highly tailored displays, organized by groups. Groups can be multi-level, with customizable dimensions, layouts, coloring, legends and standard curve display options.

Multi-analyte Data Acquisition
Assign multiple biosensor types during a quantitation experiment for a new level of versatility and increased experiment walkaway time. Screen multiple biosensor chemistries against a single analyte for biosensor selection (i.e. Protein A, Protein G and Protein L biosensors against one antibody). Or, use different biosensor chemistries to serially multiplex quantitation of an array of analytes.

QUANTITATION DATA ANALYSIS

Grouped View (Quantitation)
Simplify visual analysis of datasets with highly tailored displays organized by groups. Groups can be multi-level, with customizable dimensions, layouts, coloring, legends and standard curve display options.

X-Y Graph
Analyze trends and correlations between parameters by plotting any two numerical analysis table values in an X-Y Cartesian map. Toggle between linear and logarithmic scales, click-and-drag to customize the dimensions and copy to the clipboard for easy export.

Accelerated Binding Calculation
Process quantitation datasets faster and view final data sooner. An improved and validated algorithm provides the same quality quantitation data Octet has always produced — in a fraction of the time.

KINETICS DATA ANALYSIS

Report Points for Raw Data
Generate report points from raw data and analyze any assay step, such as baseline, loading or association within an experiment. Save the report point settings to a file and recall those settings during future experiments to streamline analysis.

1:2 Bivalent Analyte Model
Perform full kinetic analysis of data for bivalent analytes interacting with an immobilized ligand. The bivalent model provides terms for two association constants and two dissociation constants.

For more information, download the Octet Software v7 datasheet from www.fortebio.com.

Ready to upgrade? Order or request a quote online. ☑️

Why Does Academia Prefer Octet For Label-Free Protein Characterization?

The Octet RED system is a workhorse in our lab that provides reliable small molecule-protein binding kinetics and affinity data in a fast, easy label-free assay.

James Delproposto, Research Associate, Life Sciences Institute, University of Michigan

Out of the core lab, onto your benchtop
Measure concentration, kinetic and affinity constants and generate SPR-quality data right on your benchtop

Label-free kinetic characterization in a few minutes!
Complete experiments in minutes rather than days using a Dip and Read™ assay— setup requires only pipeting reagents into a 96- or 384-well microplate

Direct quantitation assays for antibodies and other proteins
Replace HPLC and ELISA with Octet to measure antibody and other protein concentrations in crude samples accurately without secondary reagents

Visit www.fortebio.com or call 888.OCTET-75 today to learn more about the Octet platform.

Fast. Accurate. EASY.
Application Focus

Agri-food Applications Using Bio-Layer Interferometry: A Proof of Concept

By Terry McGrath & Terry Fodey, Asset Technology Centre, Queen’s University Belfast

The ASSET (ASsured, SaFE and Traceable food) Technology Centre is a research centre within the Institute of Agri-food and Land Use at Queen’s University, Belfast. The vision of ASSET includes the development of innovative, state-of-the-art scientific techniques that will create a niche food forensic strength to develop a new dimension in animal and human health, food safety monitoring and traceability. Food Safety has been a research focus at Queen’s University for several years, pioneering the use of biosensor technology in the area of food safety monitoring, and the team are now acknowledged to be the world’s leading research group in this area. Sub-strand 1 of the ASSET project concentrates on the development of highly innovative biosensor-based detection systems relating to chemical contaminants found in foods.

Regulations require food to be tested for chemical contaminants such as veterinary drug residues, toxins and pesticides. Biosensors are one of the tools that are utilised for surveillance. Commercial biosensor development has mainly been driven by life sciences and drug discovery applications but all market sectors have the same basic demand: fast, accurate results. Manufacturers have tried to meet this need by developing instrumentation with higher throughput and multiplexing capabilities. Within the agri-food sector, focus has shifted from the detection of single analytes towards the simultaneous screening of families or multiple families of contaminants. Whole family screening may be possible with generic binding proteins but an alternative multiplexing approach is required for the simultaneous analysis of multiple families or multiple sample matrices. The Octet RED96 system with its Dip and Read™ biosensors is perfectly suited for this role, its 8 channels facilitating up to 96 immobilised biosensors.

Cornerstones of routine food contaminant analysis include repeatability, reproducibility and the ability of an instrument to overcome non-specific binding caused by complex sample matrices such as extracted meat samples.

Within run repeatability and between run reproducibility of the system were evaluated using the shellfish toxin domoic acid, (molecular mass ~311 g/mol), as a model analyte. For concentration determination, calibration curves were constructed using a competitive inhibition approach to maximise the dynamic range of the calibration curve. A derivative of domoic acid was immobilised on to AR (amine reactive) biosensors and a predetermined concentration of specific antibody was mixed with various concentrations of domoic acid in the microplate prior to the introduction of the immobilised biosensors. The result was a curve exhibiting decreasing signal as the concentration of analyte in the microplate increased.

Six AR biosensors were immobilised and five point calibration curves were processed simultaneously in duplicate (n = 2, typical %CV<3.2%), on each biosensor with regeneration of the biosensor between cycles. As can be seen from the plotted calibration curves (n = 6), the biosensors demonstrate very low variation in repeatability of the calibration curve (CV of midpoints = 2.8%). The reproducibility of the calibration curve was also evaluated by constructing the curves on different days (n = 3). As can be seen from the plotted curves the variation between days is also very low (CV of midpoints = 4.1%).

The ability of the technology to show inhibition in complex food matrices was also evaluated using domoic acid and an antibiotic, called chloramphenicol, which has been banned from use in food producing animals. Positive and negative samples were evaluated for five different matrix types using crude sample preparations. For solid matrices, buffer was added and the samples were homogenised and then centrifuged. For liquid matrices, they were diluted with buffer, mixed and then centrifuged. The table shows the ratio of sample (g) to buffer (mL). As is evident, all of the matrices showed a difference in signal between the negative and positive sample. This indicates that the instrument can still recognise the specific binding of antibody to immobilised derivative, despite the presence of various sample components that could potentially have interfered with the detection technology.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Spike</th>
<th>Conc. (ng/mL)</th>
<th>Report Point Signal (nm)</th>
<th>Difference (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shellfish 1:8</td>
<td>DA</td>
<td>0 50</td>
<td>1.0546 0.4326</td>
<td>0.6220</td>
</tr>
<tr>
<td>Honey 1:4</td>
<td>CAP</td>
<td>0 20</td>
<td>0.8686 -0.0562</td>
<td>0.9248</td>
</tr>
<tr>
<td>Honey 1:1</td>
<td>CAP</td>
<td>0 20</td>
<td>0.3229 -0.2020</td>
<td>0.5249</td>
</tr>
<tr>
<td>Milk (full fat)</td>
<td>CAP</td>
<td>0 20</td>
<td>0.8008 0.0374</td>
<td>0.7634</td>
</tr>
<tr>
<td>Milk (semi-skimmed)</td>
<td>CAP</td>
<td>0 20</td>
<td>1.0723 0.1665</td>
<td>0.9058</td>
</tr>
<tr>
<td>Chicken 1:4</td>
<td>CAP</td>
<td>0 20</td>
<td>1.3506 0.2701</td>
<td>1.0806</td>
</tr>
<tr>
<td>Beef 1:4</td>
<td>CAP</td>
<td>0 20</td>
<td>1.2556 0.2523</td>
<td>1.0033</td>
</tr>
</tbody>
</table>

TABLE 1: Specific detection of domoic acid and chloramphenicol in various complex matrices.
The work with domoic acid and chloramphenicol has shown that the Octet RED96 system is capable of excellent repeatability and reproducibility and can deal with the crude sample preparations associated with food contaminant analysis.

With the eight channel setup and the possibility to immobilise biosensors with different compounds the instrument facilitates multiple contaminant analysis within one run. Since no liquid is removed from the well it is entirely feasible to analyse a single sample, in a well, for multiple contaminants. With the excellent repeatability and reproducibility it is also possible to use multiple biosensors to construct the calibration curve thus freeing up more space for analysing samples. With being such a flexible open system it can support, anything from, the analysis of a single sample for multiple analytes to analysis of different sample matrices for multiple analytes.

This initial proof-of-concept work has provided very exciting results for the ASSET Technology Centre and indicates, to us, that there is the potential of new multiplexing solution for the rapid screening of agri-food contaminants.

List of Recent Publications Citing the Octet System

Soluble Periplasmic Production of Human Granulocyte Colony-Stimulating Factor (G-CSF) in Pseudomonas fluorescens

Chaperone Activity of a B-Crystallin Is Responsible for Its Incorrect Assignment as an Autoantigen in Multiple Sclerosis

Structural and Biophysical Analysis of Bst-2/Tetherin Ectodomains Reveals an Evolutionary Conserved Design to Inhibit Virus Release

Exploiting Nucleotide Composition to Engineer Promoters

Role of Iron and Sodium Citrate in Animal Protein-Free CHO Cell Culture Medium on Cell Growth and Monoclonal Antibody Production

Chronic Intranasal Treatment with an Anti-Aβ30–42 scfv Antibody Ameliorates Amyloid Pathology in a Transgenic Mouse Model of Alzheimer’s Disease

Robo4 Maintains Vessel Integrity and Inhibits Angiogenesis by Interacting with UNC5B

Ly5 Acetylation is a Widespread Protein Modification for Diverse Proteins in Arabidopsis

The Peroxisomal Targeting Signal 1 in Sterol Carrier Protein 2 is Autonomous and Essential for Receptor Recognition
CP Williams et. al. *BMC Biochemistry*, 2011, 12:12

TMEFF2 is a PDGF-AA Binding Protein with Methylation-Associated Gene Silencing in Multiple Cancer Types Including Glioma
K Lin et. al. *PLoS ONE*, 2011, 6 (4), e18608

An Insertion Mutation that Distorts Antibody Binding Site Architecture Enhances Function

For a complete list of references by year, visit www.fortebio.com/references.html.
Kinetic analysis of protein-protein and protein-small molecule interactions is a key application for real-time, label-free systems such as the Octet family of instruments. The Octet platform is currently utilized in various segments of the pharmaceutical and biotherapeutic drug development processes such as early discovery, process development, late-stage clinical trials and manufacturing/QC. Applications include quantification of protein concentrations in crude media, screening antibodies in crude supernatants, characterization of protein-protein interaction kinetics ($k_\text{on}$, $k_\text{off}$ and $K_d$), and screening small molecule fragment libraries.

In this discussion, tips and tricks for obtaining the best possible kinetic data will be presented. This will include optimization of target immobilisation and analyte binding, along with regeneration strategies (if required) and sample plate and method design. The Octet analysis software options will also be discussed, giving details of the analysis models and their applicability to data interpretation.

TARGET MOLECULE IMMOBILISATION

The most important consideration in choosing a strategy for target molecule immobilisation is the retention of biological activity.

Direct Coupling

Whilst direct covalent attachment results in stable, non-reversible target immobilisation, a number of factors need to be considered. Direct coupling is usually performed directly to the biosensor surface, such as with amine coupling. This has the potential for loss of target activity resulting from steric hindrance. In amine coupling procedures, it is difficult to restrict the number of sites on the target molecule which are linked to the biosensor surface. Potentially, any free lysine residue can be involved in the linking and if any are close to the analyte binding site, then loss of target activity can result.

The following requirements should be considered when performing amine coupling:
- The target must be pure, and must not contain any extraneous amines, or be diluted in any amine-containing buffer.
- The target must also be prepared in low salt buffer at a pH just below its pI value, to maintain a balance between creating enough charge to attract it to the biosensor on the one hand, but keep as much unprotonated lysine as possible on the other hand.
- If the target has been lyophilized from buffer, it may be necessary to perform a desalting step to reduce the buffer ionic strength.

For more information on amine coupling, refer to ForteBio’s Technical Note No. 7 Batch Immobilization of Protein Onto Amine Reactive Biosensors.

Site-directed Coupling

A site-directed approach is recommended to maximize surface activity. Favorable orientation of target on the biosensor surface can be achieved by the use of specific capture approaches aimed to couple the target via a known position or label. Additionally, steric hindrance can be minimized by the inclusion of chemical linkers.

Several oriented capture options are available on the Octet platform. The use of such a capture approach also enables immobilisation of targets from crude preparations.

A second important consideration in choosing an immobilisation technique is minimal dissociation of target from the biosensor. The most favourable in this respect are amine reactive and streptavidin biosensors, though others can be assessed for target capture stability.

The most common, and one of the most favored, capture systems employs biotin labeled target and streptavidin biosensors. This approach has a number of advantages over direct amine coupling:
- Biotinylation is performed in solution at neutral pH.
- The ratio of biotins per target molecule can be controlled.
- It is easy to incorporate a long chain linker into the biotin tag to minimize steric effects.
- The biotinylated target can be prepared in batches and re-used for multiple capture experiments. Refer to ForteBio’s Technical Note No. 10 Batch Immobilization of a Biotinylated Ligand onto Streptavidin Biosensors for more information.

ANALYTE BINDING

Analyte can be prepared in most commonly used buffer systems, but it is important that the concentration is known. Any errors in this concentration will be carried through to errors in the calculated affinity.

We recommend ForteBio’s kinetics buffer (available as a 10X solution; ForteBio Part number 18-5032) which contains PBS + 0.1% BSA, 0.02% Tween20 and 0.05% sodium azide as a sample buffer unless the particular interaction calls for another buffer composition.

OPTIMISING THE KINETICS ASSAY

Target Preparation

Biotinylate the target using either NHS-PEG4-Biotin (Pierce, part no. 21329) or NHS-LC-LC-Biotin (Pierce, part no. 21343). Label at

<table>
<thead>
<tr>
<th>Biosensor</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Human Fc Capture (AHC)</td>
<td>Human Fc tag</td>
</tr>
<tr>
<td>Anti-Murine Fc Capture (AMC)</td>
<td>Murine Fc tag</td>
</tr>
<tr>
<td>Streptavidin (SA)</td>
<td>Biotin tag</td>
</tr>
</tbody>
</table>

TABLE 1: Octet biosensors that allow site-directed immobilization of target.
a molar ratio of about 1:1 biotin:target. It is important to remove unreacted biotin reagent prior to immobilisation. This is usually performed using desalt columns such as Zeba Micro Desalt spin columns (Pierce, part no. 89877). For more information on biotinylation of target proteins, refer to the following technical notes.

- Technical Note No. 6 Biotinylation of Protein for Immobilization onto Streptavidin K Biosensors
- Technical Note No. 11 Biotinylating Antibody in Stocks Containing Carrier Protein
- Technical Note No. 12 Biotinylating Very Small Quantities of Protein for Immobilization onto Streptavidin Biosensors

**Target Immobilisation**

Initially, use the parallel processing capability of the Octet system to optimise the target concentration. Use a dilution series from 25 µg/mL to 10 µg/mL, aiming for biosensor saturation. Use the ‘extend current step’ and ‘skip to next step’ buttons during the experiment to optimise the loading times.

**Analyte Binding**

Ideally, the analyte dilution series should cover the affinity constants range of 10x\(K_D\) to 0.1x\(K_D\). If the \(K_D\) is unknown, begin with a high concentration and titrate down. For robust kinetics analysis, use at least 5 analyte concentrations, preferably in duplicate.

If the expected \(K_D\) is in the nM range, an association of 15 minutes and a dissociation of 15–30 minutes may be sufficient to obtain kinetic constants with low error.

If the \(K_D\) is < 1 nM, an association of 15–30 minutes and a dissociation of one hour or more may be necessary to obtain kinetic constants with low error.

If the \(K_D\) is unknown, use the ‘extend current step’ and ‘skip to next step’ buttons during the experiment to optimise the analyte binding times.

**Regenerating Biosensors**

The unique 8- or 16-channel simultaneous processing employed in Octet systems allows accurate kinetics constants to be measured without the need for regeneration at all. Still, in most cases, biosensors can be regenerated (removal of bound analyte) for re-use, which has favorable implications on assay costs.

For interactions of modest affinity (around 100 nM or lower), the analyte may often take a long time to fully dissociate. In such cases, if the biosensors are to be re-used, they should be regenerated. Capture biosensors such as Anti-Human Fc and Anti-Murine Fc Capture can be regenerated by removal of both analyte and target molecules. This is usually achieved using low pH, but should be optimised for each interacting pair. For more information on regenerating Anti-Human Fc and Anti-Murine Fc Capture biosensors, refer to the corresponding datasheet or technical note.

Amine reactive and Streptavidin biosensors are regenerated by removing only the analyte molecule, due to the irreversible nature of target immobilisation. For successful regeneration of biosensors, complete removal of bound analyte and retention of target activity are essential.

To optimise regeneration conditions, use the Octet system’s parallel processing to investigate 8 or 16 regeneration conditions in a single experiment. Figure 1 shows how the Octet system was used to assess 8 regeneration conditions, over 11 sequential analyte binding cycles (total assay time: 2 hours).

Using the grouping function in ForteBio’s Analysis software, rapid evaluation of each regeneration condition is possible, as shown in Figure 2.

**Reference Wells and Biosensors**

To obtain accurate protein-protein interaction kinetics, referencing-out the buffer effects are important. Include a reference sample well containing buffer in the sample plate in your experiment. For protein-small molecule interactions, include a reference biosensor to perform double referencing.

---

**FIGURE 1.** Identifying which of eight solutions is best for regeneration in less than two hours. Eleven cycles were performed with each solution for protein bound to a biotinylated receptor on Streptavidin biosensors.
Reference biosensors should ideally include an immobilized non-active protein similar to the specific target protein. Avoid BSA as the non-active protein as it is prone to non-specific binding interactions. If no suitable non-active protein is available, block the active sites on the biosensor (for example, use biocytin to block Streptavidin and Super streptavidin biosensors).

For more information on protein-small molecule kinetics on the Octet platform, refer to ForteBio’s Technical Note No. 16 Small Molecule Binding Kinetics.

**SETTING UP THE KINETICS METHOD**

A kinetics experiment requires baseline, association and dissociation steps to be set up in consecutive order. Octet data acquisition software simplifies assay set via experiment templates. Assay optimisation for pH scouting and regeneration scouting can be set up quickly using the templates.

Other factors to note are that the analyte concentrations should be entered in the software in MOLAR terms, and, to include at least one well of buffer (0 nM sample) for reference subtraction. Kinetic measurements under non-mass transport limiting conditions can typically be achieved using a shake speed of 1000 rpm.

**DATA ANALYSIS**

Choosing a model for curve fitting in the Octet data analysis software should be done on the basis of an understanding of the chemistry underlying the binding interaction. As a general rule, use the simplest curve fitting model available (1:1) unless you have prior knowledge that the interaction is more complex. Before trying a curve fit model, get as much information about the interaction under study as possible, such as stoichiometry of binding, purity of ligand and heterogeneity of analyte.

Use other curve fit models in Octet data analysis software to assess experimental optimisation. For example, the 2:1 heterogeneous ligand binding model will show if the surface contains multiple affinity binding sites. If this model gives a good fit compared to 1:1, try to optimise the target loading level further, or consider oriented capture of the target on the biosensor.

The mass transport binding model will show if the analyte is diffusion-limited at the surface. If you suspect the presence of mass transport limitation, reduce the target immobilisation level and/or increase the shake speed can be increased up to 1500 rpm.

**SUMMARY**

By careful optimisation of experimental parameters, kinetics analysis on the Octet systems will yield excellent kinetics data. Oriented, site-specific coupling of target molecule to the biosensor provides the best homogeneous surface. The optimal immobilization level of target on the biosensor should be arrived at by scouting target solution concentration and incubation time. Analyte concentration is ideally explored from $0.1\times K_D$ to $10\times K_D$ with multiple concentrations in-between. Use at least one reference sample and perform double-referencing for small molecule assays. Set up the experiment such that the interaction occurring at the biosensor follows a 1:1 binding model. Good luck with your experiments!
Tracking product integrity by measuring $k_a$, $k_d$, and $K_D$ during:
- Upstream fermentation
- Downstream harvest and purification
- Post-derivization (pegylation)
- Formulation development

AMC biosensors deliver:
- Simplified assay development — validated capture format eliminates need for biotinylation or purification of antibodies from crude matrix.
- Ease of use — a high-affinity anti-mouse antibody conjugated to the biosensor is readily available for off-the-shelf use.

Quality — a high-quality biosensor verified by ForteBio for use in kinetic analysis.

The AMC biosensors are pre-immobilized with a high affinity antibody against the Fc portion of mouse IgG. This antibody can capture and immobilize mouse IgG (mIgG) and other Fc-containing ligands to produce a stable surface suitable for interaction analysis. The capture surface is particularly suited for immobilizing mIgG from cell culture supernatants and other complex mixtures in which biotinylation is not an option.

AMC biosensors streamline the antibody screening workflow by enabling immobilization of mouse Fc-containing proteins on the biosensor directly from a crude or purified matrix. No purification or biotinylation steps are required, thereby facilitating high throughput screening methods.

Mouse IgG subtypes IgG1, IgG2a and IgG2b are recommended for use with AMC biosensors; IgG3 should be evaluated on a case-by-case basis. The AMC biosensor is ready for use on all Octet systems.

For more information, visit the AMC biosensor web page and download the AMC biosensor datasheet and technical note.

- FIGURE 1: Kinetic characterization of the interaction between a mouse IgG1 antibody and a FAb analyte at 4 different concentrations using AMC biosensors. After an equilibration step and a preconditioning cycle, the assay consists of 5 assay steps.
  - Step 1: equilibration, Step 2: loading (capture) of mouse IgG1, Step 3: baseline, Step 4: association kinetics, Step 5: dissociation kinetics. The raw data for a full assay is shown in Figure 1B. After data processing (including reference subtraction using the 0 nM trace), the association and dissociation traces were fit to a 1:1 binding model (1C, red lines). Values of $K_D$, $k_{on}$, and $k_{off}$ were extracted from the curve fitting analysis (1D).
The ForteBio Chorus Hits a High Note

By Sriram Kumaraswamy, Ph. D., Senior Product Manager, skumaraswamy@fortebio.com

ForteBio's customers were out in full force the last three months singing the octet gospel all over the world. By that I meant the Octet user community was incredibly active in the last three months, presenting their stories at various conference venues. The presenters are quite a diverse group, and the presentations covered a wide range of topics across early biotherapeutic discovery, process development, pre-clinical testing, antibody engineering, vaccine development and small molecule drug discovery. The common theme among the various presenters is that they are quite a happy bunch, going by what they have to say about the Octet platform.

Most of the presentations are available in pdf format in the LEARNING section of ForteBio's website. A few are webinars that can be played back with audio. Listen in and maybe you will get hooked on a tune or two!

Octet Technology Platform in Therapeutic Antibody Discovery and Development
Thilo Riedl
Sr.Scientist Assay & BioAnalytical Science, Genmab
ForteBio User Workshop, March 2011, Cambridge, UK

Quantitative Analysis of Influenza Hemagglutinin Binding
Peter Coombs
Postdoctoral Fellow, MRC National Institute for Medical Research
4th Annual Proteins Congress & Vaccines Congress, April 2011, London, UK
Also presented at ForteBio User Workshop, March 2011, Cambridge, UK

Use of Disposable Label-free Real-time Biosensors in the Drug Discovery of Monoclonal Antibodies
Yasmina Abdiche
Associate Research Fellow, Rinat-Pfizer
SBS 17th Annual Conference, March 2011, Orlando, USA

Fast Label-free Assay for Quantitation of a Humanized Antibody Therapeutic
Mark Dysinger
Research Scientist, Pfizer, Inc.
Cambridge Healthtech Institute Web Symposium on Advances in Label-free Assays for Quantitation and Kinetic Characterization of Biotherapeutics, March 2011
Also presented at ForteBio's 2nd Annual Workshop on Advances in Ligand Binding Assays at the AAPS National Biotechnology Conference, May 2011, San Francisco, USA

Quantitation of Recombinant Human Factor IX in Bioreactor Harvest Samples Using Bio-Layer Interferometry
Daniel Eustace*, Jennifer Manfra and Kannappan Veeraragavan
Quality Analytical Development, Pfizer Global Manufacturing
IBC Antibody Development and Production, March 2011, Washington, USA
Kinetic Characterization of Protein-Protein Interactions in Purified and Bacterial Lysates Using the ForteBio Octet RED System

Ryan Case
Senior Scientist, Amgen, Inc.

Cambridge Healthtech Institute Web Symposium on Advances in Label-free Assays for Quantitation and Kinetic Characterization of Biotherapeutics, March 2011

Bli Technology: From mAb Discovery to Product

Marijn Van der Neut Kolfschoten
Senior Scientist, Antibody Discovery, Crucell Holland B.V.

7th Annual Bioprocess International Europe, April 2011, Nice, France

CDK2 Ligand Affinity Measurements for the Community Structure Activity Resource Center (CSAR)

James Delproposto
Research Associate, University of Michigan

Drug Discovery Chemistry, April 2011, San Diego, USA

Screening and Characterization of Fully Human Antibodies from Velocimmune® Mice Using Real-Time Label-Free Interaction Analysis

Matthew Blome
Scientist, Therapeutic Proteins, Regeneron Pharmaceuticals

PEGS, May 2011, Boston, USA

Using Disposable Label-Free Biosensors to Rapidly Screen Crude Antibodies Against Solution Antigen

Yasmina Abdiche
Associate Research Fellow, Rinat-Pfizer

ForteBio’s 2nd Annual Workshop on Advances in Ligand Binding Assays at the AAPS National Biotechnology Conference, May 2011, San Francisco, USA

Fully Automated CHO Host Cell Protein Quantitation in Monoclonal Antibody Therapeutics

Dan Schuessler
Analytical Scientist, GlaxoSmithKline

ForteBio’s 2nd Annual Workshop on Advances in Ligand Binding Assays at the AAPS National Biotechnology Conference, May 2011, San Francisco, USA

Epitope Reduction Screening to Aid the Development of a PEGylated Protein Therapeutic

Sean Bell
Senior Scientist, Biomarin

ForteBio’s 2nd Annual Workshop on Advances in Ligand Binding Assays at the AAPS National Biotechnology Conference, May 2011, San Francisco, USA
July – December 2011 Events

Visit ForteBio at any of its upcoming events to learn more about the Octet platform and other products that help make your workflow fast, accurate, and easy.

- **July 23–27**
  25th Symposium of the Protein Society
  Boston Marriott Copley Place, Boston, MA
  Booth 209

- **July 25**
  Workshop: Recent Advances in Label-free Assays for Kinetic Analysis of Protein-Ligand Interactions
  Boston Marriott Copley Place, Boston, MA

- **Aug 22–25**
  CHI The Bioprocessing Summit
  Marriott Long Wharf Hotel, Boston, MA
  Booth 5

- **Aug 25**
  ForteBio's Second Annual East Coast User Workshop: Advances in Label-Free Assays for Bioprocessing, Ligand Binding, Antibody Characterization and Screening
  At CHI The Bioprocessing Summit
  Marriott Long Wharf Hotel, Boston, MA

- **Sept 20–22**
  Symposium: MipTec Basel/Switzerland
  Messe Basel, Basel, Switzerland
  Booth C41

- **Oct 11–13**
  Biotechnica Germany / CHI PEGS Europe
  Hannover Exhibition Grounds, Hannover, Germany
  Exhibition Floor

- **Oct 14**
  Inaugural Octet User Workshop and Advances in Label-Free Assays
  Park Inn Hannover, Germany

- **Oct 11–13**
  Biotechnica Germany / CHI PEGS Europe
  Hannover Exhibition Grounds, Hannover, Germany
  Exhibition Floor

- **Dec 4–8**
  IBC's 22nd Annual International Conference Antibody Engineering
  Hilton San Diego Bayfront Hotel, San Diego, CA
  Booth 206

---

**Online Ordering**

Online quote requests are available to all customers at shop.fortebio.com

Customers in the USA can order online at shop.fortebio.com. We accept VISA, Master Card and AMEX.

For more information about ForteBio's Octet System for label-free, real-time detection, applications and services, visit www.fortebio.com or contact us directly.

---

**EDITORIAL STAFF**

**Editor**
Sriram Kumaraswamy

**Assistant Editor**
Tori Muir

**Contributors**
Phil Buckle
James Delproposto
Terry Fodey
Weilei Ma
Terry McGrath
Wesley McGinn-Straub
Sriram Kumaraswamy
Chris Silva

**Art & Design**
Tori Muir and Karen J Kennedy

**Miss or misplace an issue?**
View issues online at www.fortebio.com/interactions

---

© 2011 ForteBio, Inc., ForteBio, and the ForteBio logo are trademarks and/or registered trademarks of ForteBio, Inc.