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interactions

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New Protein L Biosensor For Antibody Fragment Quantitation

by Wesley McGinn-Straub, Product Manager, ForteBio, wms@fortebio.com

Antibodies are so rich in biochemical features that nearly every piece and parcel of their structure has been harvested and put to use, leading to widespread adoption of whole molecules, Fabs, Fvs and scFvs in both medicine and biotechnology. In conjunction with the Octet instruments, ForteBio's Protein A and Protein G biosensors have accelerated protein science research and development by enabling easy, fast and accurate quantitation of whole molecule and Fc fusion proteins. However, due to the absence of an Fc domain, — to which both Protein A and Protein G bind — detection of Fab, Fv and scFv fragments on the Octet platform has required custom biosensor preparation and validation by the end user.

ForteBio's new Protein L biosensor now brings the capability for direct detection of most kappa light chain-containing antibodies to the Octet platform, providing a reliable , time-saving "out-of-box" solution for Fab, Fv and scFv fragment quantitation. The Protein L biosensor can, of course, be used to detect whole molecule antibodies that contain kappa light chains, and may be particularly useful when the target antibody demonstrates weak affinity towards both Protein A and Protein G.

Protein L, an immunoglobulin binding protein originally isolated from *Peptostreptococcus magnus*, binds human kappa I, III and IV and mouse kappa I light chains of antibodies and antibody fragments. This broad specificity allows the Protein L biosensor to recognize a wider range of antibody isotypes than either Protein A or G, including IgG, IgM, IgA, IgE and IgD. Across mouse, rat and human IgG subtypes, Protein L possesses "strong" binding to nearly all species and subtypes — whereas Protein A and Protein G show "weak" or "no binding" to several species and subtypes. The Protein L biosensor is especially useful for quantifying antibodies and antibody fragments from serum–based culture because Protein L does not bind bovine immunoglobulins, which often contaminate serum supplement and thereby generate significant background signal.

As has been done for both Protein A and Protein G biosensors, standard-sensitivity and high-sensitivity acquisition parameters on the Octet platform have been developed as starting points for assay development with the Protein L biosensor. Standard sensitivity conditions (2 minute read time, 400 rpm shake speed) generated a dynamic range of 0.5–2000 µg/mL using a human IgG Fab on an Octet RED96 system. Extending the read time and increasing the shake speed (10 minute read time, 1000 rpm shake speed) produced a dynamic range of 0.05–300 µg/mL. Using the same model system, the Protein L biosensor has been shown to regenerate at least 10 times in conditioned medium with less than 5% loss in capacity, making the biosensor a cost-effective solution for screening

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INSIDE THIS ISSUE

ForteBio Spreads its Wings — A Profitable Life Sciences Tools Company

Terry Salyer, VP Sales, ForteBio, tsalyer@fortebio.com

The end of 2010 marked the completion of my third exciting year leading ForteBio's global distribution team, and another year of sales growth among the highest in the industry. ForteBio's continued investment in building a global sales and support team has earned the company a world-class customer list with Octet platforms placed in 21 countries. Awareness of the Octet platform continues to build as ForteBio's customers publish their successful research and share their positive opinions with other scientists regarding the value of the Octet platform. ForteBio has grown rapidly from a small start-up company to a profitable solution leader within the life science industry.

After spending almost 20 years in the life sciences industry working with scientists that use detection systems, I have found customer applications to always be interesting and diverse. ForteBio's customers in pharmaceutical, biotech, academia, diagnostics, medical and government sectors use Octet systems to detect proteins and small molecule interactions in samples originating from human, animal, and plant material. The Octet platform has enabled research to be "label-free" by allowing measurement in crude unfiltered matrices and has provided the unique ability to conduct multiple experiments on a reusable sample volume. ForteBio's customers have used the Octet platform to monitor analytes as small as molecular fragments and as large as virus particles. The company has reached out to its customers for their feedback and has strengthened the Octet platform with innovative instrument capabilities and workflows, software tools, and new biosensor chemistries through aggressive product development initiatives. The company now offers five Octet instruments with varying capabilities and affordable price points, enabling access to even budget-limited laboratories.

I sincerely believe that the biggest compliment a customer can give an instrumentation company is to purchase a second instrument. The purchase of another system signifies that ForteBio's products offer a solution that has true value in the customer's workflow. As a testament to the products' usefulness and reliable performance, over one third of the ForteBio customer base have purchased two or more Octet instruments, replacing cumbersome ELISA and SPR techniques in many departments of their organizations. Many of the company's customers are upgrading to the new Octet 384 platform. In 2010, the company sold 55% more Octet 384 systems than it initially estimated for the year. The improved workflow automation and reduced sample volume requirements in the Octet 384 systems have allowed more SPR and ELISA applications to be converted to the Octet platform.

I envision 2011 to be another successful year for ForteBio, and look forward to working with new customers that will fuel future product innovations. I would like to thank all current customers for their business, feedback and loyalty.

Technical Tip:

Kinetic Analysis of Membrane Protein Interactions on the Octet Platform

by Wesley McGinn-Straub, Ph. D., Product Manager, ForteBio, wms@fortebio.com and
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ForteBio's Octet platform provides a label-free and user-friendly solution for detecting molecular interactions with a unique microplate based workflow that offers flexibility and throughput. The assay format has been used widely to quantitate, screen and kinetically characterize soluble protein and small molecule analytes. However, its applications to membrane proteins (which govern most cellular input and output signals and represent the largest class of pharmaceutical drug targets), are relatively few, primarily due to the technical difficulties in acquiring homogeneous, natively folded membrane protein samples.

In a collaborative effort, ForteBio and Integral Molecular have combined technologies to generate a powerful new solution for assessing membrane proteins as analytes in a label-free assay. Using Integral Molecular's lipoparticle, a unique nano-scale expression system, natively folded membrane proteins can be generated in a format that is highly amenable to analysis on the Octet platform. The protein product is cell-free and does not require detergent extraction or resolubilization. The lipoparticle technology re-engineers membrane-enveloped retroviruses to produce non-infectious virus-like particles (VLP) that contain the membrane protein of interest (Figure 1). The lipoparticles are approximately ~150 nm in diameter and the membrane protein of interest can be enriched up to 100-fold as a percent of total protein.

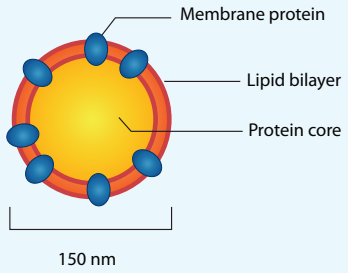

FIGURE 1: A membrane protein of interest expressed into a lipid bi-layer lipoparticle, prepared using a proprietary technology from Integral Molecular and ready for *in-vitro* interaction analysis. The dense protein core of the lipoparticle provides a biologically relevant context at the inner membrane surface.

To demonstrate the potential of the ForteBio Octet and Integral Molecular lipoparticle technologies, the integral membrane protein chemokine receptor type 4 (CXCR4) was expressed in a lipoparticle and its interaction with an anti-CXCR4 antibody was analyzed on an Octet RED instrument. The lipoparticle was immobilized by exploiting the high affinity of carbohydrates displayed on its surface for a wheat germ agglutinin (WGA)-coated biosensor. The association between the lipoparticle-displayed CXCR4 receptor and the anti-CXCR4 antibody, as well as the dissociation event (Figure 2), were recorded in real time. The raw binding data was processed

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Technical Tip

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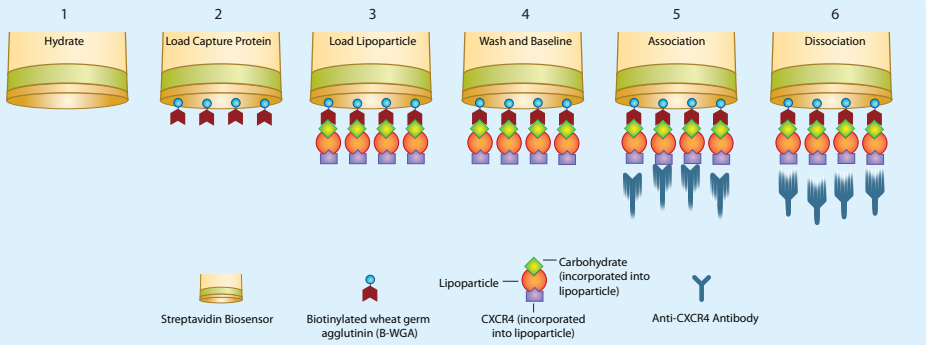


FIGURE 2: Example workflow for kinetic characterization of the interaction between the CXCR4 membrane protein and an anti-CXCR4 antibody. Step1: Streptavidin biosensor hydration, Step 2: Load biotinylated wheat germ agglutinin, Step 3: Immobilization of the CXCR4-displaying lipoparticle, Step 4: Baseline in buffer, Step 5: Measure association kinetics between anti-CXCR4 antibody and lipoparticle-displayed CXCR4, Step 6: Measure dissociation kinetics between anti-CXCR4 antibody and lipoparticle-displayed CXCR4.

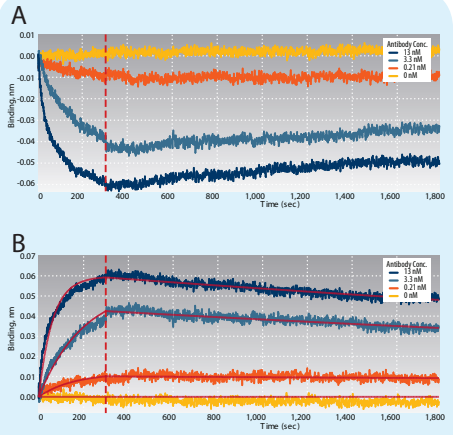
and fit to a 1:1 binding model, providing k_a, k_d and K_D values for the interaction between the integral membrane protein CXCR4 and the anti-CXCR4 antibody.

The presence of the lipoparticle on the surface of the biosensor placed the interaction surface approximately 150 nm away from the biosensor surface. Similar to effects observed on most optical biosensor technologies, this large distance caused an expected inversion of the data; the observed amplitude change was a *negative* nm shift rather than a *positive* nm shift (Figure 3A). The negative data is otherwise identical to "normal" data, but it is not compatible with curve fitting algorithms. ForteBio's Octet data analysis software offers a "flip" feature that inverts the data, thereby enabling curve fitting and kinetic constant derivation (Figure 3B, 3C).

To validate the process of obtaining kinetic data from inverted binding curves, a model system was generated that observed the same protein-protein interaction with and without a lipoparticle (inverted data and normal data, respectively). Building on the example that used lipoparticle-displayed CXCR4 and the anti-CXCR4 antibody, the interaction between the mouse anti-CXCR4 antibody and donkey anti-mouse antibody was utilized.

As expected, the amplitude shift observed on the lipoparticle was *negative* whereas the amplitude shift observed off (i.e., without) the lipoparticle was *positive*. After applying the "flip" function to the raw data acquired on the lipoparticle, both datasets were processed and fit to a 1:1 binding model to obtain values for k_a, k_d and K_D. Kinetic data for the interaction observed on (inverted) and off the lipoparticle were in excellent agreement, indicating that the signal observed on the lipoparticle, although inverted, is otherwise identical to "normal" data and that processing such data with the ForteBio "flip" function provides valid kinetic results.

A more detailed description of the use of lipoparticles on the Octet platform will soon be available in an application note on ForteBio's website.



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K_D (nM)	k_a (1/Ms)	k_a error (1/Ms)	k_d (1/s)	k_d error (1/s)
13.3	9.88 E5	9.34 E3	1.32 E-4	1.33 E-6

FIGURE 3: Association and dissociation of lipoparticle-displayed CXCR4 and an anti-CXCR4 antibody. After correction of the signal using a reference biosensor, the processed data was negative and therefore not amenable to curve fitting (3A). Application of the ForteBio software "flip" function inverted the data (3B), allowing fitting with a 1:1 heterogeneous ligand fitting model (red lines) and derivation of quantitative kinetic data (3C).

The synergistic potential of label-free assays using natively folded integral membrane proteins opens important avenues for basic research and drug development efforts. Not only can membrane protein receptors such as GPCRs, ion channels and integrins be screened against libraries of small molecules, antibodies and phage, but the interaction of lead candidates with the receptor can be kinetically characterized. Since binding kinetics differentiate potential medicines and contribute to efficacy, safety and pharmacodynamic/pharmacokinetic parameters, this information can be critical to key decision-making steps during candidate selection and drug development.

The authors acknowledge Joseph Rucker at Integral Molecular Inc. for contributing technical data to this article.

Protein L Biosensor

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experiments. Because the interaction of each antibody with Protein L is different, results can vary with both analyte and matrix.

The Protein L biosensor can be used for a wide variety of applications in the antibody laboratory. During cell line development, it can be used to determine the productivity of pools and individual clones. During process development, rapid determination of antibody concentration using the Protein L biosensor and the Octet platform expedites the iterative process of optimizing growth conditions and culture media. The Protein L biosensor can also be used in formulation development and quality control of biologics.

For more information, visit the Protein L biosensor web page and download the Protein L datasheet and technical note. To request a quote for Protein L biosensors, visit the ordering page.

Generating Reliable Kinetic Data for Protein-Ligand Interactions

by James Delproposto, Research Associate, University of Michigan, delpropo@umich.edu

The Community Structure Activity Resource (CSAR, www.csardock.org) group is developing a database of high quality protein-ligand structures and the corresponding binding affinities. The data will be provided from in-house experiments and community collaborations. The proteins are generally well-studied structures that have been targeted in drug discovery projects. Current projects include CDK2, LpxC, and urokinase. The drug-like ligands for each of these targets consist of several series of compounds with a wide range of affinities.

Current docking and scoring models rely on experimental data, which is typically incomplete: crystal structures of protein-ligand complex may exist, but affinity data is not available. Affinity data for a series of compounds might be published, but the crystal data may be poor quality or incomplete. Variations between experimental conditions can create differences between published data. Without high-quality datasets, training these models becomes difficult if not impossible. Our goal is to create complete high-quality data sets to assist computational scientists with their goals.

To populate the affinity database, three label-free methods are used: the Octet RED system, a thermal denaturation assay, and isothermal titration calorimetry (ITC). These produce both complementary and overlapping data. The methods use dissimilar technologies to independently produce affinity values (K_D) with exactly the same compound, protein, and buffer. This helps to reduce experimental error and gives modelers more confidence in the accuracy of the data.

The Octet RED system determines rate constants, k_a and k_d; these are used to calculate a K_D, defined as k_d/k_a. We determine K_D for 6 different compounds in 2 hours using 6 concentrations for each compound and 0.1 mg of protein. After scouting runs to determine optimal concentrations of compound, pre-made compound plates allow us to complete up to 4 runs in a single day. This throughput has dramatically increased the data we can produce in a short time, turning the Octet RED system into the workhorse of the CSAR project.

Compound	Octet RED (Average)			ITC (Average)		ΔG, Calculated Average	
	K_D (M)	k_a (1/Ms)	k_d (1/s)	K_D (M)	ΔH (kcal/mol)	From ITC	From Octet RED
CS1	3.32E-05	3.57E+04	1.09E+00		-7.52		-6.11
CS2	1.84E-06	2.22E+05	3.81E-01	4.23E-06	-8.76	-7.33	
CS3	8.08E-07	3.35E+05	2.59E-01		-17.70		-8.31
CS4	2.12E-05	7.19E+04	1.29E+00	6.03E-06	-7.95	-7.12	
CS9	5.83E-05	2.35E+04	2.58E-01		-12.70		-6.50
CS10	9.10E-07	7.97E+04	6.66E-02				-8.24
CS11	2.80E-07	2.81E+05	8.40E-02	1.41E-06	-11.75	-7.98	
CS13	9.65E-07	6.95E+04	3.94E-02				-8.21
CS14	1.43E-06	6.43E+04	7.70E-02				-7.97
CS15	5.57E-07	1.13E+05	3.67E-02				-9.00
CS16	7.73E-08	2.94E+05	1.69E-02				-9.70
CS17	5.39E-08	1.22E+05	3.40E-03				-10.16
CS18	9.61E-07	1.00E+05	8.23E-02				-8.21
CS19	2.29E-07	1.75E+05	2.01E-02				-9.06
CS20	7.77E-07	8.59E+04	5.62E-02				-8.33
CS241	2.87E-07	175033.3	4.97E-02				-8.92
CS242	5.28E-07	3.21E+04	1.73E-02		-6.57		-8.56
CS244	1.79E-07	9.73E+04	1.28E-02				-9.20
CS245	2.29E-07	9.72E+04	8.20E-03				-9.06
CS246	5.26E-07	1.14E+05	5.92E-02	1.94E-07	-14.99	-9.15	
CS247	2.26E-08	9.18E+04	2.30E-03				-10.40
CS248	1.06E-06	7.84E+04	7.97E-02				-8.15
CS260	9.86E-08	1.32E+05	1.33E-02	1.79E-07	-10.70	-9.20	
CS261	2.51E-07	3.97E+04	1.00E-02	1.28E-06	-3.39	-8.04	
CS262	4.73E-08	6.49E+04	2.61E-03				-10.00

Note: Data obtained for CDK2 binding to compounds of molecular weight ranging from 193 Da to 509 Da. All experiments were performed at T = 298K.

The thermal denaturation assay is performed on the Thermofluor® platform. It is a high-throughput method that detects changes in fluorescence as the protein is heated and melts. Ligand added to the protein stabilizes it and increases the melting temperature. The difference between the melting temperatures of the protein and the protein-ligand complexes allows calculation of an affinity value. A run takes about 2 hours, and uses a comparable amount of protein and compound to the Octet RED system. The K_D values, however, must be calculated using the Van't Hoff equation.

Some of the variables used for the equation are difficult to obtain and must be estimated, therefore the affinity value calculated from the melting temperature difference is an estimation at best. The dye used to create the fluorescent signal also has the potential to interact with the protein in unforeseen ways. An article recently published indicated that the 1,8 ANS dye binds allosterically to CDK2, causing a conformation change. This unexpected challenge invalidates our current Thermofluor data and requires us to repeat experiments with a new dye.

The third method we use is ITC. It has a long history of being a reliable method

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Engaging Customers — A Passion at ForteBio

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ForteBio is strongly engaged with its customers and other protein researchers in many ways. The company's field applications and sales teams constantly interact with customers throughout the world on a daily basis, often helping them optimize their use of the Octet platform in their work. ForteBio meets researchers involved in bioprocessing, drug discovery and protein research at various conference venues and interacts online with them via webinars to promote knowledge of the Octet platform as a broadly useful analytical method.

ForteBio was present at the **Antibody Engineering and Therapeutics** conference last December, and at the **Peptalk** event earlier this year, both held in San Diego. ForteBio conducted exhibits and presented a poster authored by Dr. Yasmina Abdiche of **Rinat/Pfizer** on "Use of Label-free Biosensors in Epitope Binning and Mapping of Monoclonal Antibodies" at both events. The poster describes the use of Octet 384 systems for fast and efficient binning and mapping experiments and compares the BLI and SPR techniques to highlight the advantages of the Octet platform for these applications. A copy of the poster can be downloaded from ForteBio's website.

Brian Miller of **Biogen Idec** presented a seminar at Peptalk on "Using Dip and Read™ Label-free Assays for **Rapid Antibody-Antigen Characterization**". Brian discussed the adoption of Octet systems in Biogen Idec's protein engineering platform and how the Octet has become an **indispensable tool** for determining antibody titer for research-scale production, quickly screening protein libraries expressed in *E. coli* for molecules displaying improved biophysical properties and rapidly performing cross-blocking assays to group antibodies into similar bins.

On December 1, 2010, **Genetic and Engineering News** presented an educational **webinar** to an audience of 230 listeners on "Advances in Label-free Assays for Antibody Discovery, Development, Characterization, and Bioprocessing". Dr. Yasmina Abdiche of Rinat/Pfizer, Dr. Arnout Gerritsen of **Genmab BV** and I covered a number of applications on the Octet platform, with comparisons to SPR and related technologies wherever appropriate, including protein-protein interaction kinetics, epitope binning and mapping, antibody titer determination in cell line development, downstream process monitoring, low-affinity anti-drug antibody monitoring and bioprocess contaminant detection assays. A **recording** of the webinar is available for viewing at www.fortebio.com/webinars.html.

Also on ForteBio's webinar web page, visitors will find recordings of other webinars presented by ForteBio scientists in November, 2010. During three well-attended events, ForteBio's scientists discussed the utility of the Octet platform in kinetic analysis of protein-protein and protein-small molecule interactions, immunogenicity monitoring and bioprocess contaminant testing.

In Spring 2011, ForteBio is reaching out again with five **new** webinars between February 17 and March 22. Each webinar will cover a different set of applications. Recordings of these webinars will be available for viewing on the company's website. Site visitors can also access many user presentations, posters, application notes and much more at www.fortebio.com/literature.html.

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and gives affinity data very similar to that produced by the Octet RED system. ITC has a number of advantages and disadvantages. It is the slowest of the three methods, but unique in that it can provide accurate enthalpy of binding values. Each run uses several milligrams of protein that must be saturated with 2 to 3 times the molar equivalent of compound. In addition, it can measure only a very limited range of affinities. For the CDK2 project, the affinities of only six of twenty-seven compounds could be used, due to issues with ligand solubility and affinity range. The enthalpy alone was determined for an additional four compounds. This data can be combined with the affinity data from the Octet RED system to determine the Gibbs free energy and entropy of binding.

Consistent results are the most important aspect of using the Octet RED system. Data must be consistent between runs as well as between different methods. The data the Octet RED system has provided has been very consistent with ITC. In cases where it has been possible to use all three methods on a compound, the data produced by each method is similar (as a general rule, an affinity within 3-fold of one determined via another method is considered identical). Some of the inconsistencies in our data may be due to compound solubility issues. The Octet RED system requires far lower ligand concentrations than ITC, and thus is less affected by compound solubility issues. Thermal denaturation assays are often used for compounds with low solubility, but still require saturation of the protein. The Octet RED can produce usable data at concentrations ten- to one-hundred fold lower than ITC and Thermofluor, helping ensure accurate compound concentration in solution.

The first data set for the CSAR database is nearing completion. The next projects have begun and have already presented unique challenges requiring the use of all the tools we have available to provide consistent and reliable affinity data. CSAR will continue to create high-quality protein-ligand structures and affinity datasets to aid the computational chemistry community.

Thermofluor is a trademark of Johnson and Johnson. The ITC measurements were made with the Nano ITC Low Volume system from TA Instruments.

Accurate Measurement of Kinetic Rate Constants and Affinities of Viral Proteins and Cell Signaling Interactions

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

ForteBio's bio-layer interferometry (BLI) technology enables label-free, real-time analysis of biomolecular interactions on the Octet platform. Octet systems measure signal changes that are directly proportional to changes in thickness and density of the binding layer at the interaction interface. BLI is thus capable of accurately measuring kinetic rate constants and binding affinities for any specific interaction. Accurate measurements require careful implementation of thoughtful experiments designed to obtain high-quality data.

The Octet platform is currently used for kinetic screening and characterization in a variety of different applications ranging from **small molecule-protein** to **antibody-"virus-like-particle"** (VLP) interactions. The publications below describe how the Octet platform serves the broad life sciences research community by providing reliable kinetic information on biomolecular binding interactions.

Scott F. Michael et. al. measured the affinity of inhibitory peptides binding to viral coat proteins of the dengue virus in their study of the DENV E glycoprotein as a target for rational inhibitor design.[1] In another study, human monoclonal antibodies produced against **dengue virus E proteins** were studied on the Octet platform to determine their functional properties and to rank their affinities.[2]

In a separate study, Octet systems were used to elucidate the mechanism of action in signaling. Bosanac et. al. utilized binding **measurements on the Octet platform** to **complement crystal structure data** in elucidating the role of Hhip protein in hedgehog signaling, which is crucial for many aspects of embryonic development.[3,4] **Octet kinetic information matched closely** with data produced in **Isothermal Titration Calorimetry (ITC)** experiments. Kinetic data obtained on the Octet platform was also utilized to demonstrate that peptide activators of pro-HGF b stimulate Met receptor signaling in the absence of proteolytic processing.[5]

Bourhis et. al. at Genentech describe insect cell expression and purification of soluble human LRP6 extracellular domain and its use in screening Wnt/β-catenin signaling inhibitors. Wnt/β-catenin signaling is initiated on the cell surface by association of secreted Wnt with its receptors Frizzled (Fz) and LDL-receptor-related protein 5/6 (LRP5/6).[6] Studies of these molecular interactions have been a significant technical challenge because the proteins have not been available in sufficient purity and quantity. Direct binding assays using the **Octet RED system revealed the existence of multiple, independent Wnt and Dkk1 binding sites** on the LRP6 co-receptor, suggesting new possibilities for the architecture of Wnt signaling complexes and a model for broad-spectrum inhibition of Wnt/β-catenin signaling by Dkk1. Additionally, they discuss the use of **Octet RED system to screen antibodies blocking LRP6 function** and predict their cellular activity based on the subset of Wnt they inhibit.[7]

Prischi et. al. at the National Institute of Medical Research (NIMR) and other collaborating institutions describe the use of a combination of biophysical methods to define the structural bases of the interaction of a bacterial ortholog of frataxin, an essential iron-binding protein (reduced expression levels of this protein are sufficient to induce Friedreich's ataxia, a currently incurable neurodegenerative disease) with the IscS/IscU complex, involved in the iron-related metabolic pathway.[8] The **kinetic** information obtained as part of the study on the **Octet** platform is described to be in **excellent agreement** with **Isothermal Titration Calorimetry (ITC) results.**

In a publication authored by researchers at the NIMR and other institutions, the Octet platform is used to measure **DNA-protein binding** interaction affinities to study the role of the far upstream element (FUSE) regulatory system in the upsurge of c-myc levels during the cell cycle.[9] The c-myc promoter's misregulation has reportedly been correlated with a broad range of cancer pathologies.

Synaptotagmins and SNAREs are known to couple Ca^{2+} sensing to membrane fusion during Ca^{2+}-triggered exocytosis, but unraveling the mechanism of this coupling has proven extremely difficult. A recent study provides crucial insights into the nature of synaptotagmin-SNARE interactions and reveals unsuspected similarities between synaptotagmins and viral fusion proteins.[10] The interactions between SNARE complex and Syt3 protein are probed using a combination of single-molecule fluorescence microscopy (smFRET), x-ray crystallography, pull-down assays and Octet binding assays. The authors report **good correlation** between **affinities** measured using the Octet platform and those observed via **smFRET distributions** as well as those obtained with **pull-down assays**.

Binding affinities of **unmodified** and **conjugated antibodies** for **cytokines** were monitored on the Octet platform in a study of polysaccharide-antibody conjugates as cytokine-neutralizing agents.[11] Octet data showed that conjugating high molecular weight sugar to antibodies resulted in constructs that retained their cytokine binding affinities, and also demonstrated measurable differences in dissociation kinetics between the different constructs — indicating that conjugation can strengthen or weaken affinity.

These are a few examples in which the Octet platform is being used to generate accurate kinetic and affinity measurements. Researchers are able to take advantage of the unique capabilities of the Octet Platform such as Dip and Read biosensor format, fast time-to result and **non-destructive sampling** to measure kinetic parameters and produce reliable results in complicated sample matrices. To learn more about the capabilities of the Octet platform for kinetic analysis, please visit www.fortebio.com/kinetic_char.html.

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For a complete list of references by year, visit www.fortebio.com/references.html.

March – May 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.

- **Mar 16–18** **IBC Antibody Development & Production**
  Hyatt Regency Bellevue, WA
  Booth 402
- **March 23** **End User Workshop / UK**
  Doubletree by Hilton Cambridge
  Cambridge, Cambridgeshire, UK
- **Mar 23–25** **BIT Life Sciences' 3rd Annual International Congress of Antibodies**
  China National Convention Center, Beijing, China
  Booth 36
- **Mar 27–31** **SBS 17th Annual Conf & Exh Advancing the Science of Drug Discovery**
  Gaylord Palms Resort & Convention Center, Orlando, FL
  Booth 500
- **Apr 4–5** **4th Annual Proteins Congress & Vaccines Congress (Oxford Global)**
  Copthorne Tara Hotel, Kensington London, UK
- **Apr 6-7** **7th Annual BioProcess International Europe (Informa UK/IBC)**
  Nice Acropolis Des Congress, France
  Booth 1
- **Apr 12–14** **CHI Drug Discovery Chemistry**
  Hilton San Diego Resort & Spa, San Diego, CA
  Booth 13
- **May 9–13** **CHI PEGS**
  The Sheraton Hotel, Grand Ballroom, Boston, MA
  Booth 201
- **May 16–18** **AAPS National Biotechnology Conference**
  Hilton San Francisco Union Square, San Francisco, CA
  Table 23

ForteBio is pleased to announce its presence at the following conferences, and offer *Interactions* readers a discount when they register to attend! Register using key code FB11 to claim a $200 (commercial)/$100 (academic) discount.

CHI Drug Discovery Chemistry, Apr 12–14, 2011, Booth 13

CHI PEGS Summit, May 9–13, 2011, Booth 201

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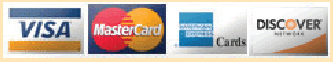

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