

GEN Drug Discovery

Innovations Mark Biosensor Development

Advances Improve Quality of Information for Compound Interactions and Screening

K. John Morrow Jr., Ph.D.

Detection and analysis of the structure and function of biomolecules is an essential step in the development of new pharmaceutical products and their application to disease conditions. Pharmacology and molecular biology research have been dramatically affected in recent years as a result of advancements in biosensor development. Of particular interest are the current investigations of label-free biosensors, which promise to revolutionize the study of critical disease-related molecules.

From a broad perspective, biosensors are platforms that employ specific binding of biological molecules to analytes in a sample in order to monitor physiological activities. Since they measure events directly, they do not require a reporter element such as a fluorescent tag that would confirm the presence of the detector molecule. Biosensors can provide information concerning molecule kinetics such as binding affinities that constitute vital information in the design of new pharmaceutical agents.

A number of companies are exploring new designs, improvements, and upgrades for their biosensor technologies. Some of these will be presented at the Society for Biomolecular Sciences' conference in April.

Label-Free Instrumentation

Biacore, now part of GE Healthcare (www.gehealthcare.com), is an acknowledged leader in the field of surface plasmon resonance (SPR) technology, having pioneered its development as a label-free means of measuring molecular interactions.

While the original technology was decidedly not user-friendly, the company has continuously upgraded its products over the years, and new hardware and software make it much more accessible as a means of studying molecular interactions. There is a strong bibliography of peer-reviewed studies using the Biacore T100 and Biacore A100 systems.

Since their introduction, they have proven their performance capabilities with the major biotechs including Genentech and Biogen Idec as well as a large number of academic institutions. According to the company, the instruments have been able to reduce costs in biotherapeutic development, advance early screening of hybridomas for mAb selection, define serum antibody responses in immunogenicity studies and immunotherapeutic development, and perform large-scale functionality studies, providing high-quality information-content interaction studies in proteomics.

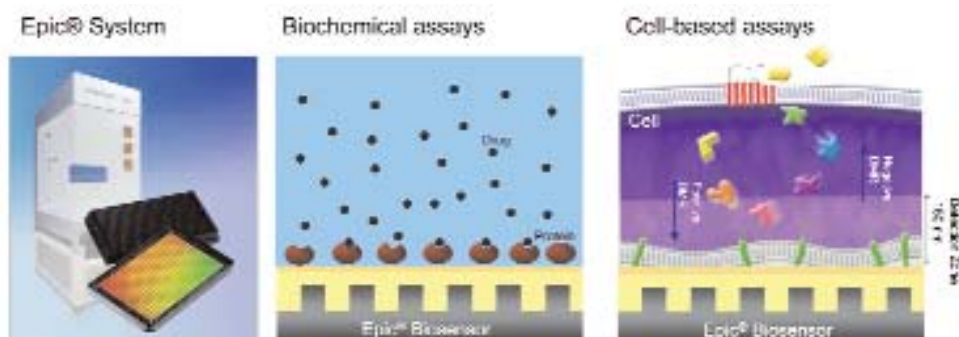
"As drug discovery paradigms have shifted from the target-directed to a systems biology approach, optical biosensors have seen increasing use in cell-based assays," explains Ye Fang, Ph.D., a scientist in biochemical technologies at Corning (www.corning.com).

Optical Tools

In optical biosensors, a biological molecule such as an antibody is bound to a surface, and when a ligand attaches to the immobilized protein, a change in the properties of a reflected light beam occurs that can be recorded by a photo cell. The information gained can define the nature and properties of the binding event and aid in the development of drug-based therapies.

Optical biosensors, however, can also be used to study the contact and binding of whole cells to ligands, with the cells bound to the surface and their interactions with drug candidates noted. Corning scientists are evaluating two optical biosensor platforms, surface plasmon resonance and resonant waveguide gratings. Both technologies take advantage of beams of light focused on the sample, which consists of cells on a thin layer of gold bound to a glass slide.

When the light beam strikes the gold, electron charge waves are created and the



Corning's Epic® system (left) is a label-free optical biosensor system that exploits evanescent waves to measure the direct binding of a drug to the protein targets immobilized on the surface of a biosensor (middle) or to measure stimulus-induced dynamic mass redistribution (DMR) of living cells within the detection zone (~150 nm) of the biosensor (right).

refractive angle of the beam is altered according to the refractive index of the material on the slide. When cells bind a particular ligand, this changes the refractive index and the resonance angle shifts.

The other alternative, the resonant waveguide grating, utilizes the coupling of the wavelength to a diffraction grating. When a beam of polarized light is coupled into a propagated wave along the waveguide, the wavelength will shift dependent on the refractive index, which is a function of the properties of the target-ligand complex.

Corning recently moved into the biosensor field by combining resonant waveguide gratings with microarray-based assays. The surfaces of 384-well microplates of the Corning Epic™ detector are modified to permit coupling of receptors or whole cells. It is also equipped with a liquid-hand-

ling system and a temperature-controlled environment and is designed to measure the ligand-induced wavelength shift of reflected light.

"Designing a resonance system adapted to 384-well microplates was quite a challenge," says Joydeep Lahiri, Ph.D., research director at Corning. "With a cell-based assay, we're not measuring a single parameter but rather an orchestrated movement or shifting of proteins within the cell. This can provide us with an understanding of how drugs affect the global picture of the cell's activities, which we could never obtain from measuring a single pathway."

According to Dr. Lahiri, with the Epic technology the resonance sensor measures events within a range of a couple of hundred

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News Discovery & Development Briefs

Takeda Commits Over \$1B for Japanese Rights to Amgen Clinical Candidates

Takeda (www.takeda.com) has agreed to pay about \$1.177 billion for Japanese rights to 13 Amgen (www.amgen.com) molecules, one of which is included as an option. Additionally, Takeda plans to acquire Amgen's Japanese subsidiary, Amgen KK.

Takeda will pay \$200 million in cash upfront. Amgen will receive up to \$340 million in development costs, \$362 million in milestones, and double-digit royalties on sales in Japan. Takeda will also become Amgen's worldwide partner for oncology candidate motesanib diphosphate. Amgen will obtain an additional \$100 million upfront, \$175 million in milestones for the first two indications, and double-digit royalties on sales in Japan.

Amira Inks \$425M Deal with GSK Related to Respiratory and Cardiovascular Diseases

Amira Pharmaceuticals (www.amira

www.gsk.com) and GlaxoSmithKline (GSK; www.gsk.com) will jointly develop, manufacture, and commercialize FLAP inhibitors for the treatment of respiratory and cardiovascular disease.

GSK will be granted an exclusive, worldwide license to all Amira's FLAP inhibitors. Amira could receive up to \$425 million in upfront and milestone fees. In addition, Amira will be entitled to tiered royalty payments based on worldwide net sales and commercial sales milestones.

Seaside Proffers \$4.5M for Fragile X Syndrome Research at Vanderbilt

Seaside Therapeutics (www.seasidetherapeutics.com) granted a \$4.5 million contract to Vanderbilt University Medical Center to discover compounds to suppress the manifestations of fragile X syndrome.

Vanderbilt researchers will use medicinal chemistry, molecular biology, pharmacology, and efficacy studies to discover and develop drug candidates. Seaside may select compounds from the collaboration for clinical development.

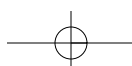
Karo Bio and Zydus Cadila Enter Inflammatory Disease Partnership

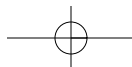
Karo Bio (www.karobio.com) and Zydus Cadila (www.zyduscadila.com) are working together to discover and develop selective glucocorticoid receptor modulators for the treatment of inflammatory diseases.

Karo Bio will use its expertise in nuclear receptor drug discovery, including structural biology, drug design, and compound characterization. Zydus Cadila will initiate a focused drug discovery and development program. Both parties will equally share the costs and rewards from this partnership.

ArQule Receives \$3M from Kyowa for Initiation of Oncology Trial

ArQule (www.arqule.com) obtained a \$3 million milestone payment from Kyowa Hakko Kogyo (www.kyowa.co.jp). The fee was triggered by Kyowa's initiation of a Phase I dose-escalation trial in Japan with ArQule's lead anticancer agent, ARQ 197, a small molecule, selective inhibitor of the c-Met receptor tyrosine kinase. ■





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nanometers, which means that it can detect movement of proteins close to the cell membrane where kinase-based responses and GPCRs are acting.

Dr. Fang and his associates have used the Epic system to study cell response following ligand binding. For example, when GPCRs bind ligands, there is a profound redistribution of cellular contents that is ordered, directional, and dynamic. Much of this activity occurs at the base of the cell and generates a dynamic mass redistribution signal, or a shift in

the resonance wavelength. This enables the investigator to carry out a systems biology analysis of the process, which takes into account its profound physiological consequences for cell function.

With this technology, moreover, drug candidates behaving as agonists or antagonists can be evaluated by high-throughput screening, with the result that many unsatisfactory choices can be eliminated early in the project.

Dr. Fang believes that the recent improvements in



ICx Nomadics
most recent offering is the **SensiQ Pioneer**.

instrumentation and assay design portend well for the expansion of high-content and high-throughput screening against a broad variety of targets such as EGFRs and GPCRs.

In regards to fragment-based screening, “small molecules have numerous advantages when it comes to shutting down a protein,” notes Daniel Elanson, Ph.D., of **Sunesis Pharmaceuticals** (www.sunesis.com). “If you could screen for very small molecules and then combine them productively, you could avoid having to screen millions of compounds in order to discover a hit.

“This is analogous to the situation in which you screen all possible three letter words versus screening all six letter words to attain a hit. Since there are many more six letter than three letter words, it is obvious how laborious such a search for really large binder molecules would be. But, if you pick out the best three letter words and then assemble them into a larger entity, you can arrive at your goal much faster.”

The problem is that these small molecules will have low affinity, and it is not obvious how you would combine them to generate the particular key to fit the complex lock of the receptor molecule in question.

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Select the miRNAs you want.

- hsa-let-7c
- hsa-let-7d
- hsa-let-7e
- hsa-let-7f1
- hsa-let-7g
- hsa-let-7f1
- hsa-miR-1
- hsa-miR-100
- hsa-miR-108
- hsa-miR-105
- hsa-miR-10a
- hsa-miR-10b
- hsa-miR-193a
- hsa-miR-193b
- hsa-miR-142-3p
- hsa-miR-16a
- hsa-miR-16b
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- hsa-miR-23a
- hsa-miR-23b
- hsa-miR-26
- hsa-miR-27a
- hsa-miR-27b
- hsa-miR-28
- hsa-miR-902b*
- hsa-miR-90a-3p
- hsa-miR-90a-5p
- hsa-miR-128
- hsa-miR-127
- hsa-miR-128a
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- hsa-miR-194
- hsa-miR-195
- hsa-miR-199a*

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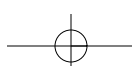
Build your own customized miRNA array with our online Custom Array Builder (CAB) at www.luminexcorp.com/CAB

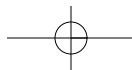
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Label-Free Biosensors for Molecular Screening		
Company	Product	Technology
Akubio www.akubio.com	RAP+id4	Acoustic biosensor
Bio-Rad Laboratories www.bio-rad.com	ProteOn XPR36	Optical biosensors
Cantion www.cantion.com	Cantilevers detected through fiberoptics	Microbeam imprinted with antibody deflected when it binds antigen
Corning www.corning.com	Epic™	Optical biosensors
DeltaDOT www.deltadot.com	High-performance capillary electrophoresis with label-free intrinsic imaging	Electrophoretic separation using algorithms to identify biomolecules
EcoChemie www.ecochemie.nl	AutoLab Espirit	Surface plasmon resonance
ForteBio www.fortebio.com	Octet System	Interferometry
GE Healthcare www.gehealthcare.com	Biacore brand products (including A100, T100, and chips)	Surface plasmon resonance
Graffinity www.graffinity.com	RAISE platform	Surface plasmon resonance for fragment library screening
GWC Technologies www.gwctechnologies.com	SPRImager®II	Surface plasmon resonance
ICx Nomadics www.nomadics.com	SensiQ	Surface plasmon resonance
Johnson & Johnson www.jnj.com	ThermoFluor	Solvachromic dye as indicator of protein unfolding
Lumera www.lumera.com	Proteomic processor	Surface plasmon resonance
MicroCal www.microcal.com	VP-ITC system	Differential scanning calorimetry
Vivactis www.vivactis.com	MiDiCal	Microplate differential calorimetry





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One approach to telescoping the laborious search for fragment binders is that developed by Graffinity (www.graffinity.com). "Our company has pioneered an efficient fragment-based discovery paradigm for the identification of high-quality hit structures that act as starting points to rapid lead optimization," states Mathias Woker, CBO.

The Graffinity team defines "fragment" in this context as molecules with a molecular weight in the range of 100 to 300 daltons and a lower structural

complexity compared to traditional HTS compounds. The number of such low-complexity compounds is vast. Combined with a label-free detection system, Graffinity offers companies the opportunity to screen a fragment library consisting of 23,000 diverse structures. Small molecule candidates can then be further optimized, since initial screening usually identifies binding affinities in the micro- to millimolar range. Label-free surface plasmon resonance imaging allows the Graffinity team

to screen proteins of interest against chip-based libraries comprising 9,216 sensor fields in a single array.

Once fragments are identified, there are a number of steps that must be taken to move candidates into lead compound status. From here on, the potency of the fragment motifs is increased in a stepwise process with the help of strategies such as fragment evolution or fragment linking. A number of simultaneous structure-based design modifications allow for efficient generation of novel compounds, reports Woker.

According to Graffinity data, the microarray-based screening of a large fragment library generates broad and deep structural affinity data from a range of derivative molecules, guiding subsequent optimization of the compound to nanomolar affinities. According to Woker, the company has employed SPR imaging to build successful inhibitors with nanomolar potency in a number of cases within a time frame of four to six months.

"Our goal is to provide a range of cost-effective systems that provide high-quality biosensor data," says Ron Gulka, director of bioinstrumentation sales and marketing at ICx Nomadics (www.discovernsiq.com). The company's most recent offering is the SensiQ® Pioneer, a fully automated,



LevTech and ATMI have merged to become the market leader for disposable mixing, high purity film and powder handling processes.



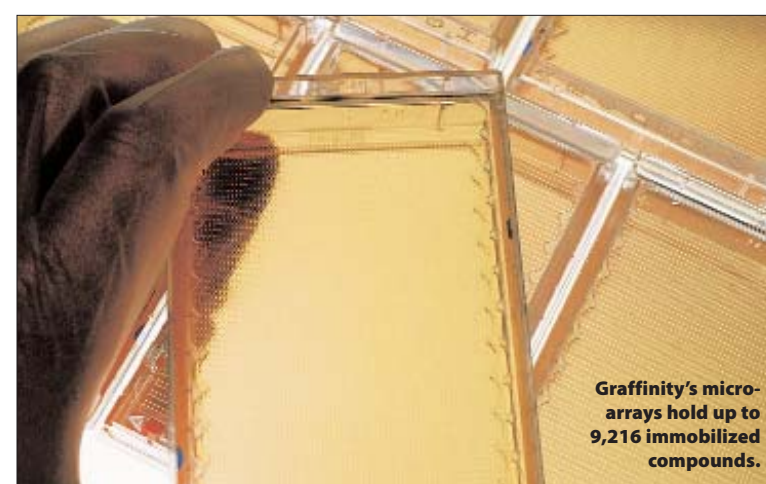
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U.S. Patent Nos. 6,441,215; 6,495,222; 6,444,413; 6,758,628; 6,837,713; 6,820,454; 7,053,323; 7,054,770; 7,240,280; 7,287,470 and other patents pending.
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high-quality SPR system. The system is configured for the characterization of biomolecular interactions including antibody selection and screening, drug discovery, and binding specificity.

According to Gulka, the industry is driven in large part by the demands of large-scale drug discovery programs carried out by big pharma. But, there is also an unrealized need for instruments appropriate for research labs with more modest requirements and smaller budgets. "We initially introduced the SensiQ semiautomated system for labs that were processing two to three samples at a time."

The Future of Biosensors

Jonathan S. Daniels and Nader Pourmand, Ph.D., of Stanford University, are among the large coterie of academic biosensor researchers. The two men believe that there is no fundamental reason why electrical readout cannot achieve the same sensitivity as optical readout. They state, however, that "there has been no systematic improvement in reported detection limits during the past 15 years of label-free, affinity biosensor research for electrical readout."

In addition, they believe that in order for electrical impedance biosensors to advance, investigations should be targeted toward applications that leverage the electrical readout advantages without requiring extreme sensitivity.

There are significant challenges that need to be resolved in order for label-free technology to be accepted in a clinical setting, regardless of the readout method. These include poor selectivity in the presence of crude samples, a need for basic science research in the area of affinity interaction changes, and a lack of workable instrumentation for sensor arrays as well as handheld, point-of-care applications.

While label-free biosensors allow assessment of real-time kinetics of binding affinities without the need for complex labeling protocols, they are notoriously hobbled by high levels of nonspecific binding in complex samples. This greatly lowers their appeal for clinical diagnostic tests, in which tissue, serum, and feces may be the starting material.

There exists today a wide gap between academic research and commercial application in biosensor technology that, if resolved, will open important new areas of commercial product exploitation. In the meantime, biosensors continue to be a valuable research tool. **GEN**

