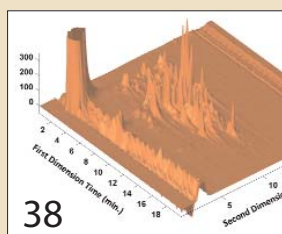




42 Due Diligence Essential in Selecting CMO

The goal should be to find the perfect fit, not just the service provider with the best credentials.



38 Maximizing HPLC Power and Throughput

Ultrahigh pressure LC, micropellicular particles, and high temperature have pushed the technique forward.

> Are Biotech Inventions Still Protectable?

With the future of patents in question, new commercial incentives are emerging. **p. 8**

> Changes Looming in Hepatitis C Arena

A bevy of companies with late-stage HCV therapeutics are racing to get to market first and claim lion's share of revenue. **p. 16**

> Microwave Chemistry Speeds Up Discovery

GEN interview explores how technology can be leveraged in life science industry. **p. 24**

> Surmounting siRNA Delivery Obstacles

Nanoparticle carriers and noninvasive methods are helping take the field to a new level. **p. 30**

Multiplexing Advances Redefine HTS

Novel Instruments, Comprehensive Services, and Streamlined Assays Boost Scientists' Efforts

K. John Morrow Jr., Ph.D.

Multiplexing is a critical component of rapid screening technologies. At the annual meeting of the Society for Biomolecular Sciences held recently in Phoenix, a number of industry and academic scientists explained their pursuit of novel technologies that allow assays to be performed simultaneously on each well of a microtiter plate. Label-free technologies such as surface plasmon resonance (SPR) are available through various companies, and several contract research organizations are adept at their application.

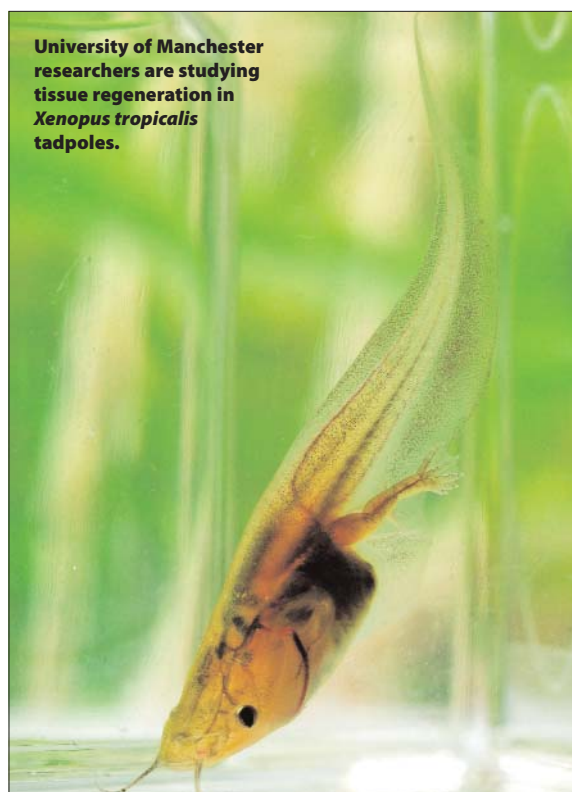
Helena Nilshans, senior market director for life sciences at GE Healthcare (www.gehealthcare.com), talked about the Biacore™ 4000 LMW extension package, which she reported “is mainly designed for small molecule drug discovery applications. Backed up by dedicated

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Scientists at the Puget Sound Blood Center developed an on-chip purification method to quickly regenerate back to the capture agents on the chip surface of Bio-Rad Laboratories' ProteOn platform for surface plasmon resonance (SPR) analysis.



Promise of Regenerative Medicine Closer to Reality



University of Manchester researchers are studying tissue regeneration in *Xenopus tropicalis* tadpoles.

Cutting-Edge Research Seeks to Expand Range of Applications for Reparative Technology

Susan Aldridge, Ph.D.

The regenerative medicine field is a hotbed of innovative research. The recent “Repairing the Body” conference, sponsored by Cranfield Health, showcased some of the cutting-edge work being done within industry and academia. The topics discussed ranged from immunological intervention to stem cell and other reparative therapies.

High-content screening is capable of embracing the biological complexities inherent in stem cell applications, according to Edward Ainscow, Ph.D., associate director of AstraZeneca's (www.astrazeneca.com) advanced science and technology laboratory (ASTL).

Regenerative medicine is a focus area for the company's new opportunities group, and ASTL's high-content

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Sticky ends

► OriGene Technologies and Institute for Systems Biology say they will create proteotypic PeptideAtlas and develop single reaction monitoring/multiple reaction monitoring mass spec standard database for 5,000 human proteins... ► Affymetrix reports it delivered first custom array designed for 100,000-sample Kaiser Permanente/UCSF genotyping project to be performed using Axiom™ genotyping system... ► University College Cork and Almac formed solid-state chemistry collaboration on applying new technologies to elucidate 3-D molecular structures from powder x-ray data... ► GE Healthcare and CardioDx entered into alliance to advance and co-develop diagnostics to improve the care and management of patients with cardiovascular disease... ► MP Biomedicals and Rusnano agreed to cooperate in areas of nanobiotechnology and pharmacogenetics... ► The St. Louis-based Genome Data Center Initiative and Rutgers University Cell and DNA Repository are among recipients of funds from \$1 billion in NIH Recovery Act awards.

Multiplexing

Continued from page 1

software tools for screening of fragments and other low molecular weight compounds, Biacore 4000 supports a range of assays from screening to characterization applications such as lead optimization.”

According to Olof Karlsson, Ph.D., a senior scientist in the Biacore R&D division, the amount of data generated on a conventional SPR system takes days to sort out with ordinary software. But with the Biacore 4000, less than two hours are required. “Not only the speed of the processing, but the overall quality of the data analysis is notably improved.”

Dr. Karlsson shared data detailing the multiplexing features of the Biacore 4000; he said that 16 targets can be run in parallel, processing up to 4,800 interactions in 24 hours. Both hardware and software are optimized for efficient large-scale assays, permitting 60 hours of unattended run time, he added. The system also includes an anti-

body-analysis package, allowing several interactions to be studied simultaneously.

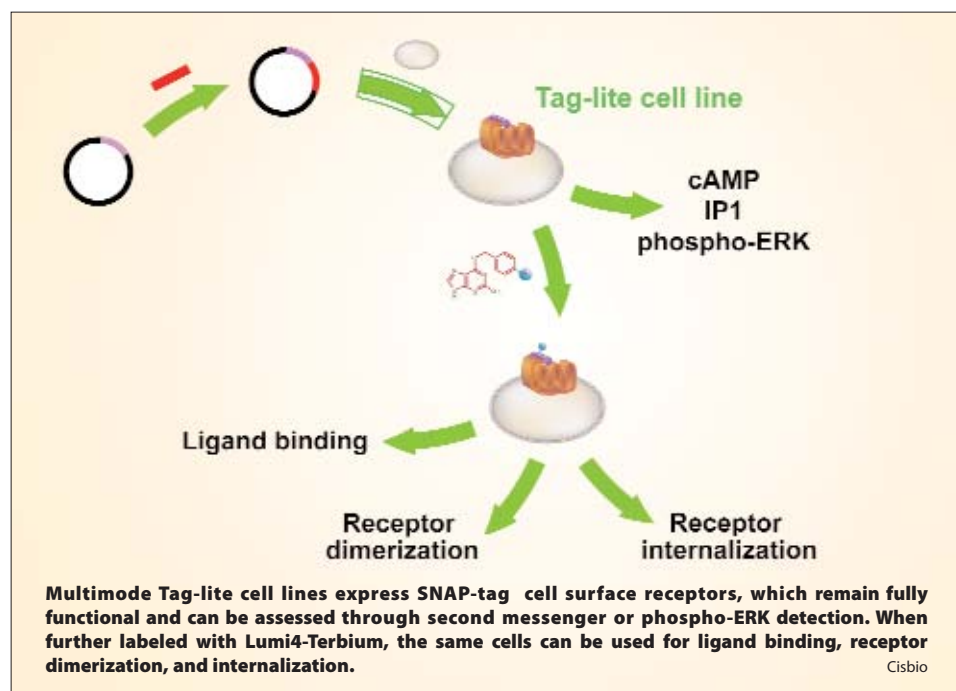
Low molecular weight pharmaceuticals are far from an endangered species. “The vast majority of drugs are still small molecules, whose cost and convenience guarantee that they won’t soon be replaced by biologics. Biacore technology allows us to use information concerning specific structures, expanding the description from a small fragment into a model of a larger molecule, optimized for binding to its target.

“The software provides parameter analyses and identifies deviations. Elimination criteria start with the new data from a run and eliminate the bad data first, allowing us to promptly focus on the good candidates.”

Focusing on Targets

BioFocus (www.biofocus.com) offers a range of discovery services, principally aimed

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News DISCOVERY & DEVELOPMENT BRIEFS

Abbott Steps Up Presence in Emerging Markets through Deal with Zydus Cadila

Abbott Laboratories (www.abbott.com) inked a licensing and supply agreement with Indian firm Zydus Cadila (www.zyduscadila.com) for at least 24 pharmaceutical products that Abbott will commercialize in 15 emerging markets. The company also reported the creation of a stand-alone established products division, which will work to expand the market for its branded generics beyond the U.S. and particularly into emerging markets.

Under the Zydus agreement, Abbott has an option to add more than 40 Zydus products to the collaboration. The deal currently includes medicines for pain and cancer, as well as cardiovascular, neurological, and respiratory diseases. Product launches are expected to begin early 2012.

GC-Rise and JS Bio Pharm Sign Separate Agreements for MediGene's Genital Warts Treatment

MediGene (www.mediGene.de) granted GC-Rise (www.gc-rise.com) and JS Bio Pharm separate licenses for the commercialization and marketing of its U.S.-approved topical treatment for genital warts, Veregen®, in China and South Korea. MediGene stands to earn development- and approval-related milestones, as well as royalties.

GC-Rise will take on clinical development of Veregen in China and has responsibility for all regulatory and approval processes in its home country. In return, MediGene will receive an undisclosed up-

front payment and milestones on initiation of the first clinical trial in China. The firm will also profit from the supply of Veregen to GC-Rise. Market launch in China is expected at the end of 2013, the firm says.

Under the Veregen deal, JS Bio Pharm will be responsible for all regulatory processes in South Korea. MediGene will receive a sales-related milestone and royalties and will also supply the drug to its partner. Launch of Veregen in South Korea is expected during 2012.

Vectura Bags \$7.5M as Novartis Starts Phase III Development of COPD Therapy

Vectura (www.vectura.com) received a \$7.5 million milestone payment from Novartis (www.novartis.com) on the latter's start of Phase III development with its combination bronchodilator, QVA149, in patients with chronic obstructive pulmonary disease (COPD). The drug is a once-daily dry powder bronchodilator comprising a fixed dose of Vectura's long-acting beta2-agonist, QAB149 (indacaterol), and the long-acting muscarinic antagonist, NVA237 (glycopyrronium bromide).

The Phase III program includes two separate year-long international trials. The first will compare the effects of treatment using either QVA149 or NVA237 monotherapy on the rate of exacerbations in nearly 2,000 patients with severe or very severe COPD. The second placebo-controlled trial will evaluate the long-term safety and tolerability of QVA149 in 339 patients.

The drug has since been launched in Germany, Ireland, and Denmark. Vectura and Novartis say they remain on track to

file additional data requested by FDA for U.S. regulatory submission during the second half of 2010.

Meda Negotiates Rights to Adeona's Phase II Fibromyalgia Drug with \$2.5M Up Front

Meda (www.meda.se) is paying Adeona Pharmaceuticals' (www.adeonapharma.com) \$2.5 million up front for the latter's flupirtine, a treatment of fibromyalgia currently in Phase II. The exclusive sublicense gives Meda rights to develop and commercialize the treatment in the U.S., Japan, and Canada. Flupirtine is already approved and sold in a number of non-U.S. markets for the treatment of pain.

Meda will take over all development and commercialization costs for flupirtine as a treatment for fibromyalgia in its licensed territories. Adeona could receive another \$5 million on NDA filing with FDA and \$10 million on marketing approval in the U.S. Meda estimates the U.S. market for fibromyalgia will be near the \$1 billion mark when flupirtine reaches the market.

Crucell Receives \$110M UNICEF Contract for Quinvaxem Pediatric Vaccine

Crucell (www.crucell.com) landed a \$110 million contract with UNICEF to supply Quinvaxem®, its pediatric vaccine, to the developing world. Quinvaxem is a fully liquid pentavalent DTwP-HepB-Hib vaccine that does not contain thimerosal, according to the company. It protects against diphtheria, tetanus, pertussis, Haemophilus influenza type b infection, and hepatitis B.

Quinvaxem is reportedly ready for use without further preparation steps by healthcare workers in the field.

This latest award brings the total value of contracts awarded since the launch of Quinvaxem at the end of 2006 to \$910 million.

From 2006–2009 as part of the Extended Program for Immunization, over 130 million doses of Quinvaxem have been delivered to more than 50 countries. Crucell has been increasing its production capacity over the last few years in order to meet the growing demand for pediatric vaccines from the developing world.

Micromet Gets €5M in Multiple Myeloma Antibody Deal with BI

Micromet (www.micromet.com) will receive €5 million (approx. \$6.4 million) up front in cash from Boehringer Ingelheim (BI; www.boehringer-ingelheim.com) as part of the companies' global collaboration to develop a BiTE® antibody against multiple myeloma. Under terms of the deal, Micromet retains U.S. co-promotion rights to any relevant product developed. The firm could also earn an additional €50 million in development and regulatory milestones, plus royalties on future sales by BI outside the U.S.

The collaboration will involve BI and Micromet working together on discovery and preclinical development of the myeloma BiTE antibody candidate. BI will then take over worldwide responsibility for clinical development, manufacturing, and commercialization of the product, subject to its partners' U.S. co-promotion rights. Micromet will also shoulder certain costs associated with preclinical activities. ■

Multiplexing

Continued from page 18

at target validation and developing molecules against targets, according to Doris Hafenbradl, Ph.D., senior director of biology and natural products. The company just reported the availability of four biologically focused libraries containing drug-like compounds that are aimed at kinases and protein-protein interactions. These compounds are modeled to bind to the kinase hinge, with allosteric sites, or to enter into other unsp-

ified binding modes on the molecule.

"We use surface plasmon resonance extensively in our screening platforms," Dr. Hafenbradl explained. "This is especially relevant for screening of our diverse fragment library in which label-free technologies are especially convenient.

"We are also well-positioned for designing drugs using our chemo-informatics library of 900,000 compounds. Using high-

throughput screening we have identified a large range of novel targets." The use of radioactive tags is no longer a popular strategy in molecular analysis, but it has to be the starting point. "If you have an enzyme that is suspect as a candidate for a therapeutic role, you can find compounds that will bind to and inhibit it."

BioFocus also maintains an active screening program for epigenetic targets. Epigenetic

modifications of the DNA form the basis of much phenotypic expression, and a wide range of disease states are now known to be associated with a group of modifications to DNA and the histone chromosomal proteins that include histone phosphorylation, methylation, acetylation, ribosyl transfer, ubiquitinylation, and proteolytic-driven cleavage.

In this case, BioFocus uses an "intelligent selection" of library subsets for primary screening, rather than employ a large compound library, which would be more appropriate for an unknown target. The narrowing process uses in silico modeling of the enzymatic site in accordance with ADME. These parameters allow the 3-D structural information concerning the target to be maximized.

"We are gradually accumulating a body of experience that will allow the elimination of futile compounds," noted Dr. Hafenbradl.

A case study on histone kinase detailed the use of a targeted library of 60,000 members, which resulted in 82 validated hits after several rounds of verification. According to Dr. Hafenbradl, this project established the validity of the firm's fast-track approach to novel chemical entities capable of inhibiting epigenetic targets.

In addition to their ability to uncover drug targets, BioFocus screening platforms have the potential to uncover disease biomarkers that could be the subject of multiplex analysis, using label-free detection such as SPR, Dr. Hafenbradl concluded.

Cellular Responses

Genetix' (www.genetix.com) CellReporter™ system was developed for assess-

SmartPlex Platform

Officials at **Thermo Fisher Scientific** (www.thermo.com) say the company's new SmartPlex platform shifts microarrays to a 96-well configuration, thus evolving toward an ELISA-like format that increases the speed and volume of multiplex applications. Because it conforms to SBS microplate standards, SmartPlex is amenable to processing thousands of samples per day, according to Jim Clements, consulting scientist at Thermo Fisher Scientific.

"The benefits of glass for immobilizing biology are widely known. Glass is a well-understood, stable material that doesn't leach materials or 'crawl' like most plastics," notes Clements. "What you place on glass today will still be there months from now."

The glass substrate is provided as a uniformly flat, clean (phillic) surface. In addition, the glass can be chemically modified with amino, epoxy, aldehyde, other silane, a nitrocellulose film matrix, or metalized.

"Because the glass and plastic can be assembled by the researcher, it can be printed on or processed prior to assembling it into a 96-well microtiter plate upper structure for additional multiplexing or processing

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ing cellular responses, according to Jerry Williamson, president of U.S. operations. The technology is based on the use of fluorescent labels that mark production of proteins by individual cells or clones of cells cultivated in soft agar.

This technology can be used to study a variety of cell functions such as cell-cycle analysis, effects of cytotoxic compounds on cell function, and the process of protein translocation.

Through the use of flexible image-analysis software, each object can be characterized, and individual cell responses can be identified. A number of assays can be run simultaneously for multiplexing functions.

Tobin Dickerson, Ph.D., of Scripps Research Institute, is using a reconfigured CellReporter instrument to isolate compounds that block the toxic effects of botulinum neurotoxin. "The cell-based assay is an excellent format to analyze inhibitors of botulinum neurotoxin."

This extremely potent toxin is fatal to humans at a dose of 1 ng/kg. Its mechanism of action is through inhibition of exocytosis in neurons. Rather than outright killing, the toxin acts by internalizing and brings transmission within the nervous system to a grinding halt. The search for an effective small molecule inhibitor has been unsuccessful since most drug screens search out compounds that prevent cell death.

As part of his approach, Dr. Dickerson screened a library of small molecules for allosteric modulators, as well as direct inhibitors. The screening system he uses reportedly works in a precise fashion, and his team has isolated a number of small inhibitory molecules. Two of these compounds are

now being evaluated in animal models.

Detection System

Jas Sanghera, Ph.D., commercial director of TTP LabTech (www.ttplabtech.com), introduced the company's new technology. "Mirrorball, which is based on an array of mirrors, will complement our existing product line."

The Mirrorball configuration was developed as a result of input from the antibody discovery industry. The instrument is based on flow cytometry because of its ability to detect low-abundance antigens, including cell surface proteins. According to Dr. Sanghera, until now laser-scanning cytometers have not been able to provide the necessary sensitivity required for mix-and-read assays.

"Mirrorball's microplate cytometric technology make this an effective system for high-throughput antibody screening. While simultaneous laser scanning ensures that Mirrorball has the requisite multiplexing and analytical capabilities, the laser-scatter channel provides an independent method for cell- and bead-based identification. This design permits improved sensitivity when multiplexed with fluorescent reporters.

"As the pharma industry is moving to biologicals as new drug entities, a rapid data-analysis platform is increasingly important. The Mirrorball scans the entire well, producing a true representation that allows you to see how the cells are being distributed. No other instrument can do that."

Receptor Investigation

Rapid and sensitive assays for cellular receptors are in demand for both clinical

Mirrorball is a new laser scanning microplate cytometer that TTP LabTech says it designed with the needs of the antibody discovery industry in mind. It is reportedly sensitive enough to be able to detect low abundance antigens, works with the mix-and-read assay format, and can perform assays in a microplate format.



and basic science applications. Cisbio (www.htrf.com) has developed a line of products based on FRET.

Tag-lite assays use a relatively undifferentiated cell line that provides a wide-ranging foundation for measuring cellular function, according to the company. The SNAP-tag labeling procedure (New England Biolabs) is used to couple a cryptate fluorophore to the receptors on the cell surfaces. Addition of a ligand carrying the second fluorophore will result in a powerful signal, forming the basis of this homogeneous assay, which requires no washing, much like ELISA-based procedures.

Tag-lite has been engineered for highly selective ligand binding, receptor dimerization, and functional assays. Among recently developed products are Cellul'erk, for measuring phosphorylated-ERK1/2, and the IP One Tb assay, for detecting inositol(1)phosphate, a major product of the phosphatidyl inositol cascade.

"For a given receptor, the same Tag-lite cell line can be used as a starting basis for these tests, which have all been streamlined and validated with pre-labeled frozen cells," stated Francois Degorce, director of marketing and communication.

"Therein lies the breakthrough of the Tag-lite concept—enabling receptor investigation to address multiple angles while eliminating the need to develop different cells for each."

Biomolecular Interactions

John Kulman, Ph.D., a principal investigator at the Puget Sound Blood Center (PSBC; www.psbc.org), described his investigations aimed at further enhancing the performance of Bio-Rad Laboratories' (www.bio-rad.com) ProteOn™ XPR-36 System. The device is designed for drug discovery, with a workflow that allows it to simultaneously monitor 36 interactions in real time.

"SPR is largely underutilized," Dr. Kulman argued, "due to cost and time limitations for producing high-quality ligands, difficulties in regenerating the ligand-coupled surface between runs, and, finally,

throughput limitations inherent in the instrumentation."

Dr. Kulman said that if these limiting factors were removed from the experimental design, quantitative evaluation of the mechanism of action of a drug candidate could be much more easily integrated into a drug-development program.

"Our approach is a blend of basic science and industrial engineering design, the end result of which is much faster and cheaper throughput without compromising data quality."

In order to simplify the workflow, Dr. Kulman developed a calcium-dependent immunocapture platform. The approach employs ligands generated by small-scale transient transfection of mammalian cells that are purified on-chip, so there is no upstream purification of ligand. Rather, the process of plasmid generation is the only rate-limiting step in ligand production. Since regeneration back to the capture agent is achieved cleanly and rapidly at millimolar concentrations of EDTA, the same capture agent can be used continuously for multiple experimental runs.

At this time, the ProteOn is not certified for clinical diagnostic applications, but Dr. Kulman believes that the throughput capacity of the instrument makes it ideal for clinical labs that process multiple patient samples. Toward this end, he and his colleagues at the PSBC are developing new diagnostic applications for a spectrum of bleeding disorders collectively known as autoimmune thrombocytopenias.

"By integrating multiplexed SPR with other high-throughput platforms such as multimode plate readers and flow cytometry, we can do some exciting stuff, we can cover a lot of ground, from biophysical studies to drug discovery and clinical diagnostics." **GEN**

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options," continues Clements.

"Cross-contamination is not an issue because each well is isolated both with a hydrophobic mask and a permanent adhesive to prevent well to well contamination.

"The adhesive components have been extensively tested using typical biological flu-

ids, laboratory chemicals, and buffer solutions over extended periods of time and no significant effect was found."

Clements points out that SmartPlex has been successfully utilized in microarray applications, as well as in cell-based and transfection assays. ■

According to Thermo Fisher Scientific, SmartPlex can process thousands of samples per day.

