MDL® Assay Explorer® goes to school

Stanford's High-Throughput Bioscience Center swings into high gear

MDL software solutions are playing a vital role at the Stanford School of Medicine, where researchers are developing a world-class center for high-throughput screening and the advancement of chemical biology research.

The School's High-Throughput Bioscience Center (HTBC), established in September 2003 by Professor James Chen, is enabling researchers at Stanford and other institutions to systematically and comprehensively study large numbers of genes and compounds by exploiting the latest high-throughput liquid handling and data acquisition techniques.

Researchers have recently used an MDL® Select suite of informatics and experiment management tools to screen Renal Cell Carcinoma (RCC) cell lines for sensitivity to a series of compounds.

Dr. David E. Solow-Cordero, who came to Stanford 18 months ago after working for eight years in the biotech industry, is associate director of the HTBC. He's responsible for managing the facility's day-to-day operations and assisting researchers with assay development and data management. "While pharmaceutical firms have been using high-throughput labs for about 10 years, the first academic involvement with HTS began at Harvard University about seven years ago," said Solow-Cordero. "In the past two to three years its use has accelerated in academia, and many major educational institutions now run HTS centers."

Though assay development in academia is now more common, its scope differs somewhat from that in industry, according to Solow-Cordero. "Most pharmas will only go after a target if there's a $300 million/year drug at the end of the pipeline, which invariably involves extremely high throughputs. Where research companies might have 10 to 20 scientists working on a project, an academic lab is more likely to have just a couple of people."

Researchers have recently used an MDL® Select suite of informatics and experiment management tools to screen Renal Cell Carcinoma (RCC) cell lines for sensitivity to a series of compounds.

The MDL® Select advantage

MDL Select workflow tools can be tailored to meet the specific needs of researchers in small to mid-sized biopharmas and academia, enabling them to take advantage of the same scalable discovery informatics solutions employed by global R&D organizations.

Academic labs are less intent on screening a million compounds a week, he says. "If an academic lab does a couple dozen screens a year, and 10 years later one of them leads to treatment of a rare disease or parasite that the pharmaceutical industry has not invested in, then the lab has done its job."

On the other hand, academic researchers need to follow the same stringent protocols as their colleagues in industry. This means validating results with statistical analysis and running good secondary assays to show specificity, according to Solow-Cordero. "I work hard to inject these kind of positive workflow practices from my industry experience into the assay development process at Stanford."

Up and running

Thanks to Solow-Cordero's efforts, the Stanford HTBC is now fully functional with all instrumentation purchased and installed. The compound libraries are in and formatted and the HTS databases are complete.

The Center's databases are built in the MDL® ISIS environment—augmented by MDL® Direct (data cartridges), MDL® Draw chemical drawing and rendering software and the MDL® Cheshire suite of chemical structure manipulation and analysis tools.

MDL® ChemBio AE provides chemical registration services and an integrated

(continued on page 4

(continued from page 3)

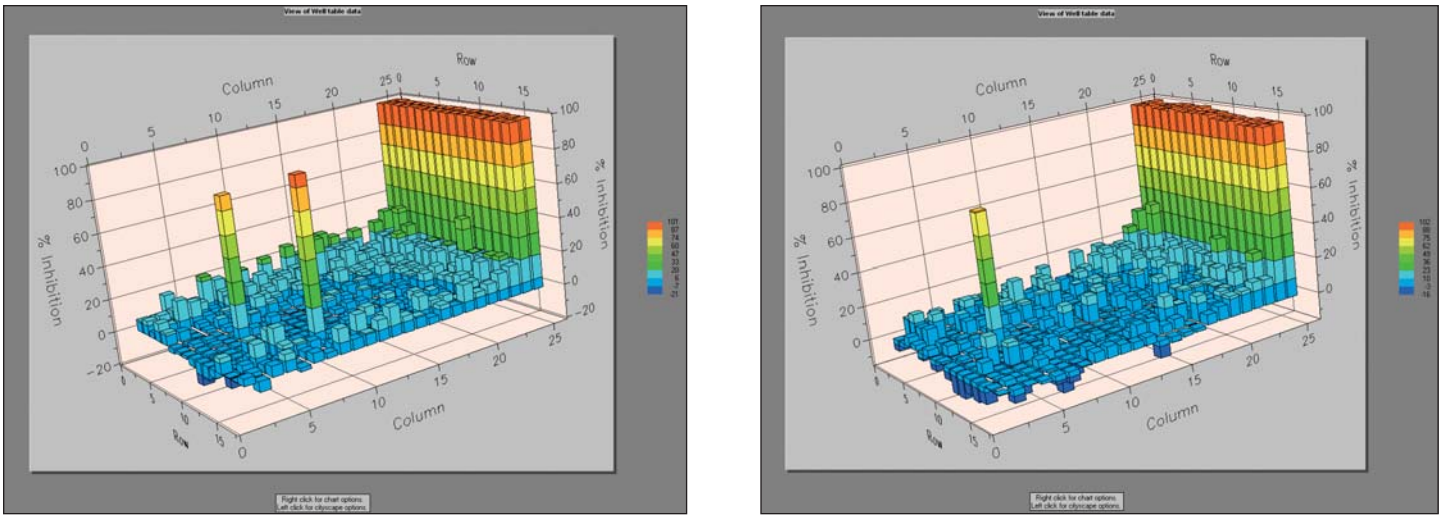


Figure 1: Cityscape views in MDL Assay Explorer showing two positive compounds in the minus cell line (RCC/VHL-) and one positive compound in the plus cell line (RCC/VHL+)

"What's great about the current MDL system at the HTBC is that it's fully integrated, so I can go from compound to compound plate to assay plate results to reporting, all using essentially an out-of-the-box solution."

Dr. David Solow-Cordero
Associate Director
Stanford HTBC

retrieval and viewing solution for chemical and biological data. The MDL® Plate Manager system provides a fully integrated central repository for plate and sample information. Finally, the MDL® Report Manager tool lets researchers extract and organize complex HTS information into a broad range of reports for information sharing and collaboration.

The primary workhorse for managing the biological aspects of HTS is the MDL® Assay Explorer® biological data management system. Assay Explorer captures, analyzes and stores experimental results in an Oracle® environment.

"MDL Assay Explorer, the core component of our MDL database system, is currently storing all our HTS data, retest data and IC50/EC50 results," said Solow-Cordero. "We're looking forward to adding ADME/Tox and *in vivo* data in the future, and I'm also looking for ways to integrate high-content image analyses."

"When I was working in industry, I used MDL ISIS/Base and ISIS/Host, creating my own Oracle tables for HTS data analysis. What's great about the current MDL system at the HTBC is that it's fully integrated, so I can go from compound to compound plate to assay plate results to reporting, all using essentially an out-of-the-box solution."

The inaugural project

The Center's first HTS project targeted Renal Cell Carcinoma (RCC), which is fatal in 40% of the 31,000 new cases diagnosed in the U.S. every year.

The project involved the parallel screening of RCC cell lines (matched for VHL expression)

Stanford's High-Throughput Bioscience Center

Comprehensive screening of biological systems

Instrumentation available at the HTBC

The Center houses a fully automated, integrated instrumentation system for high-throughput liquid handling, high-throughput detection and high-content enzyme-/protein- and cell-based screening. The liquid-handling robot can manipulate and pipet volumes to up to 200 384-well plates, which can be stocked with various ingredients, including living cells. Researchers can then add a different gene or chemical compound to each well, simultaneously testing thousands of conditions. The integrated, multi-mode plate reader measures the wells' absorbance, fluorescence, chemiluminescence or other desired characteristics. In addition, a high-content cellular imaging and analysis system enables researchers to take digital pictures of each well using a fully automated inverted epifluorescence microscope that is capable of recording multiple fluorescent images of fixed and live cells in a few minutes.

Services at the HTBC

Utilizing the HTBC, researchers can perform systematic screens of biological systems using cDNA libraries for *in vivo* or *in vitro* protein expression, siRNA libraries for targeted gene silencing and chemical libraries for the identification of small molecule modulators of specific biological processes. The Center's compound library currently contains 64,000 compounds, but this number is expected to double in a short time. The library focuses on diversity with the expectation that researchers should be able to run any sort of assay possible, with a reasonable expectation of finding an agonist and antagonist for any

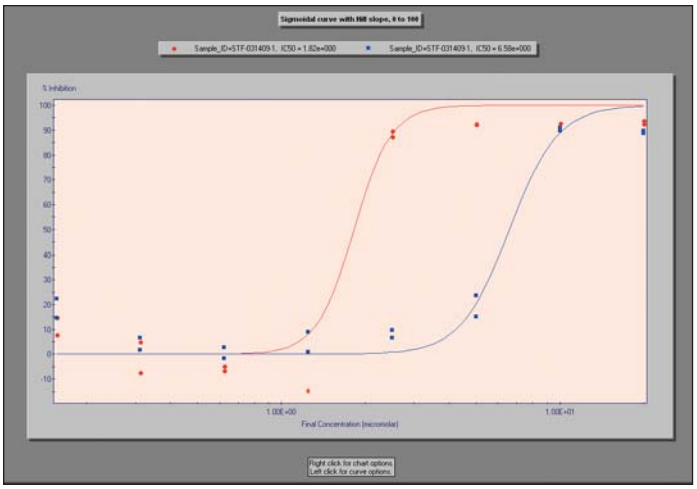


Figure 2: Dose response curves in MDL Assay Explorer showing differential in cell activity between minus and plus cell lines for a screen

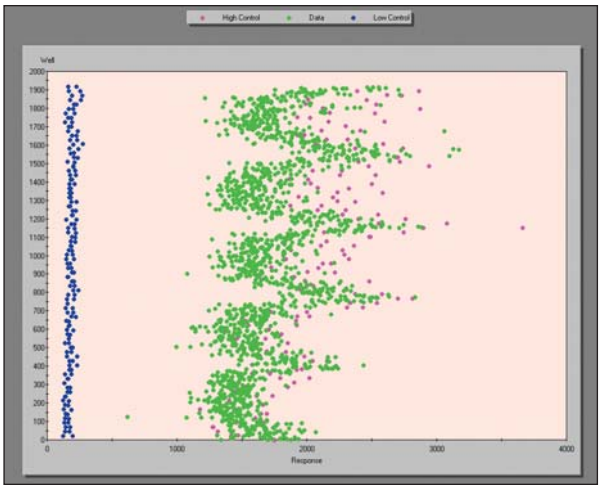


Figure 3: Scattergram in MDL Assay Explorer showing pronounced edge effects in luminescence cell-based screen (Plates 1-5, 1 on bottom)

for sensitivity to a series of compounds. Hypothesizing that the difference in transcriptional states between VHL-deficient and VHL-positive cells could be exploited for a positive therapeutic response, researchers developed a robust cellular assay and screened 64,000 compounds in 384-well plates.

MDL Assay Explorer tools enabled researchers to select actives, remove outliers, validate the data and identify trends. For example, Assay Explorer's cityscape visualization quickly revealed compounds that were specific for a cell line (refer to Figure 1), ultimately identifying 400 hits, 150 of which retested positive. Dose response curves clearly displayed the differentials in cell activity between minus and plus cell lines (refer to Figure 2). In the end, researchers found 80 compounds selective for RCC/VHL– and 17 selective for RCC/VHL+.

Assay Explorer tools were also useful in displaying trends in the data. Figure 3 is a scattergram of a luminescence cell-based screen that shows significant edge effects moving from Plate 1 on the bottom to Plate 5 at the top. This additional view of all the experimental data highlighted a potentially problematic edge-effect trend, enabling the researchers to stop the experiment, thereby saving both time and precious reagents.

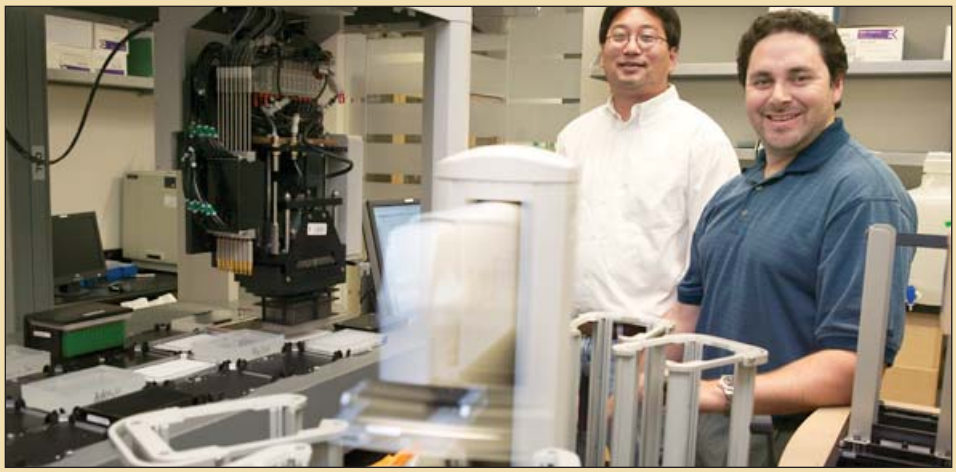
"The MDL Select software suite, anchored by MDL Assay Explorer, is central to the success of the HTBC, because its components integrate so well out of the box" said Solow-Cordero. "HTBC staff will continue to work closely with Elsevier MDL, as we build a world-class center for advancing the molecular and cellular understanding of human health and disease."■

Lab practices evolve quickly. So does MDL Assay Explorer. The 3.0 version, released in July 2005, provides increased flexibility for the scientist and contains impressive performance and integration enhancements. Tailored to biology workflows, this latest release gives biologists the freedom to add conditions and results to data analysis without having to modify templates or request administrator help.

assay. Assuming the use of 384-well micro-plates, estimated throughput performance is 100,000 enzyme assays and 50,000 cell-based assays per week. The emphasis is less on throughput than on flexibility—the ability to run many different types of enzyme- and cell-based assays in a single integrated system.

Funding of the Center

Originally funded by the Department of Molecular Pharmacology at Stanford and the National Institutes of Health, the HTBC is housed in space provided by the Stanford School of Medicine. It is expected that the Center will be self-supporting with the help of user fees, grants and donations. For more information, visit **http://htbc.stanford.edu/**.■



James Chen, Ph.D., assistant professor of molecular pharmacology, and David Solow-Cordero, Ph.D., associate director of the High-Throughput Bioscience Center, Stanford University School of Medicine, Department of Molecular Pharmacology (photo courtesy of Stanford Office of Communication & Public Affairs)