



Emory is claiming a spot as a national leader in drug discovery, on the trail to find cures for today's most challenging diseases.

# Drug Discovery

By Holly Korschun

It was 1992 and Emory pharmacologist Haiyan Fu was hot on the trail of an intriguing family of proteins called 14-3-3. Originally identified in the 1960s as exclusively connected to the brain, the 14-3-3 family had been largely ignored ever since. Fu discovered, however, that these proteins actually play a key role in regulating cell growth throughout the body, a finding that implicated them in the unchecked growth of tumor cells.

Like a cell phone company activating new handsets, 14-3-3 proteins bind to other protein targets and initiate a “conversation” that turns them on or off. Fu discovered that in cancer, this conversation can promote tumor growth by triggering the activation of proteins. These cancer-promoting proteins are like armor that allows tumor cells to resist cancer therapies as well as apoptosis, the natural process of cell death designed to eliminate damaged cells.

In hopes of halting cancer growth, Fu wanted to cut off the “conversation” from 14-3-3 to its client proteins. He began the painstaking, years-long process that would be necessary to accomplish his goal, using conventional laboratory assays. First he would need to discover exactly how the protein functioned and where it might be vulnerable to alteration. Then he would need to conduct semi-random tests that combined the protein in individual test tubes with a series of molecular compounds with potential drug-like properties.

### Birth of a drug-screening enterprise

Fast forward to 2002. Fu, along with Emory pharmacology chair Raymond Dingledine, Winship Cancer Institute associate director Fadlo Khuri, and Emory chemists Dennis Liotta and James Snyder, set their sights on securing drug discovery technology that previously had been the exclusive purview of large pharmaceutical companies—robotics equipment that could automatically handle thousands of liquids and chemical assays in minute quantities, then compute and analyze the resulting massive amounts of information. This powerful system can quickly screen hundreds of thousands of molecular compounds against protein targets.

Researchers at Harvard Medical School had become the first academic scientists to venture into this new frontier at the beginning of the 21st century. High-throughput screening equipment was becoming more affordable, and a number of chemical companies were offering large libraries of compounds for sale.

In light of Harvard’s success and Emory’s own experiences in drug development, researchers here decided it was time to claim a spot for Emory as a national drug-screening leader. Using existing research grants, combined with funds from the Keck Foundation and Synaptic Pharmaceuticals, the group began to piece together the necessary resources, including a library of 100,000 molecular compounds purchased from ChemDiv.

The Georgia Research Alliance (GRA) contributed \$400,000 for high-throughput robotics screening equipment, Amgen Corporation offered some planning funds, and the Emory University Chemical-Biology Discovery Center was officially born.

The new drug discovery center placed Emory squarely on the front lines of academic drug screening. In 2005, when the

NIH decided to fund a national network of molecular screening centers, the Emory team was already up and running. Emory was designated one of nine new centers in the NIH Molecular Libraries Screening Center Network (MLSCN) and received a three-year grant of \$9 million dollars, including a library of 100,000 additional compounds. Part of the NIH Roadmap’s “New Pathways to Discovery” initiative, the network aims to build a better toolbox for medical researchers and to serve as a resource for academic researchers throughout the country. Scientists submit suggested proteins to a national MLSCN peer-review process that picks the most promising. Each center is expected to screen the interactions of about 20 proteins a year.

Emory scientists now have two chances to submit a protein for high-throughput screening—either through Emory’s own drug discovery center or through the national MLSCN. In some cases, proteins are being screened against the compound libraries in both centers.

### Protein power

High-throughput screening (HTS) technology is one way for scientists to capitalize on the human genome project and venture into the realm of proteomics, or the study of proteins that genes produce. Scientists originally believed that each gene within the DNA sequence corresponded only to one human protein. Now, howev-

er, they recognize that the 25,000 or 30,000 genes in our DNA may encode five to 10 times that many proteins.

Because proteins play a part in everything that happens inside living cells, they are the object of intense study by scientists seeking to understand disease processes. Drug compounds are aimed at either inhibiting or enhancing the actions of proteins that are related to disease.

For example, Fu identified a tiny fragment that serves as the “mouth” of his 14-3-3 protein and enables the cell-to-cell communication that triggers cancer oncogenes. He designed an assay to connect the protein fragment to a fluorophore, a chemical marker that lights up when the protein binds to a molecule. Fu was the first Emory scientist to use the new screening equipment to test his protein against the entire library of 100,000 molecular compounds in Emory’s drug discovery center, and his protein also was selected for testing by the MLSCN network. He has identified several promising compounds that may be inhibitors to his target protein.

In conventional laboratory experiments, a researcher pipettes solutions from one test tube to another and writes down in a notebook what was done and why.

The new robotics equipment automatically pipettes liquids containing both a target protein and one of the 100,000 compounds into a tall stack of rectangular, 3x5-inch plastic “plates” containing 384 tiny holes or “wells.” A computerized plate reader analyzes the miniature chemical reactions that occur when the compounds are combined with their targets in the wells. An assay this small must elicit a simple on or off reaction—either the compound has the desired effect or it doesn’t. A program lists each of the compounds that reacted with the protein target. These compounds can then be cherry picked from the library for further study. It takes about one to two weeks to screen the protein interactions with a library of 100,000 compounds using HTS equipment.

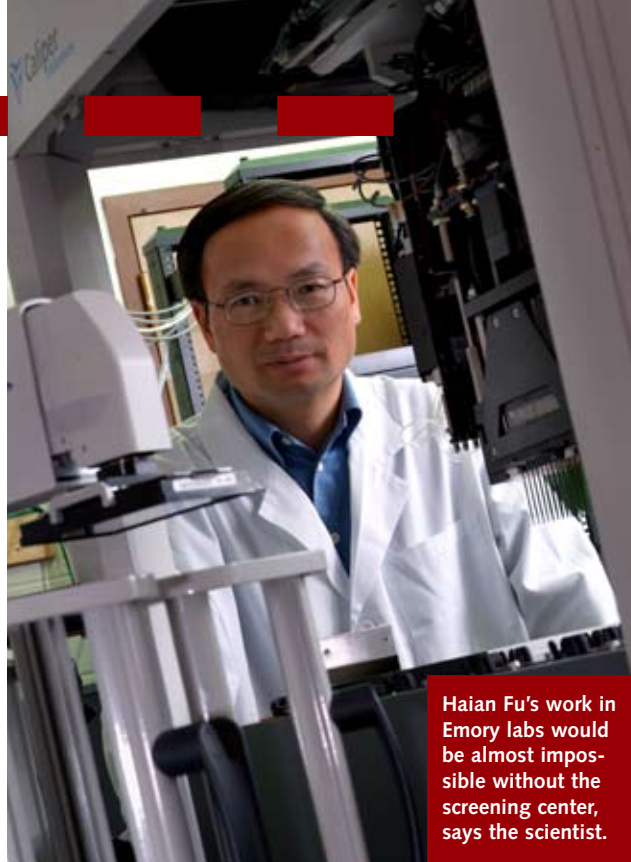
Using the cherry-picked compounds from the initial screenings, scientists like Fu then go back to their laboratories to perform a more “old-fashioned” biological experiment that sorts out the false-positive hit compounds. The end result can be up to 20 promising compounds. They then relay these compounds to

the chemistry department, where a bioinformatics and modeling group directed by Jim Snyder combines its knowledge of chemical compounds with specialized computer software to analyze the detailed molecular structures of all the hit compounds. A model of molecular activity called the QSAR, for quantitative structure activity relationship, helps predict which structural modifications of the compounds will be more active against a target. This analysis is the first step in the “hit-to-lead” process that puts a compound on the drug-candidate pathway.

Medicinal chemists use this information as a guide to remodel the structures of the validated hits by laboratory synthesis to improve the biological profiles. Fu’s lead compounds, for example, are now being optimized by a chemistry team led by Dennis Liotta. The gold standard is a compound that exerts a strong and selective effect on a target protein at a very low concentration. This means it is less likely to be toxic than a less selective, less potent compound would be. Desirable drug-like proper-



The screening center includes an automated imaging system that performs high-content screening. Instead of scientists viewing cells through a microscope, the imaging system autofocuses on one tiny well after another, reads the reaction occurring in each cell in the field, and reveals the fraction of cells that give off a fluorescent signal.



Haian Fu's work in Emory labs would be almost impossible without the screening center, says the scientist.

Haian Fu is exploring whether the 14-3-3 family of proteins supports the survival of lung cancer cells by suppressing the functions of cell death-promoting proteins. The project is one of four at Emory supported by the one of largest ever lung cancer research grants made by the NCI. Emory's grant team, which comprises 40 researchers, clinicians, fellows, and technicians from 10 departments, is exploring the most effective course of treatment for individual patients and predicting sensitivity to new and established lung cancer therapies.

ties also include high solubility in water. "A drug with zero solubility would be like ingesting a small rock," says Liotta.

Now the modeling group and the synthesis group are developing their own small library of compounds. These might include modified compounds from the screening-modeling-synthesis process. Or they might be structurally similar compounds identified by the researchers that are available for purchase but not yet part of Emory's library.

"This kind of work would be almost impossible without our screening center," says Fu. "There is a huge difference between academia and industry in screening target proteins. We design the screening assays in our own laboratories, whereas many pharmaceutical

companies often purchase ready-made assays. Academic scientists know these targets inside out because we've been studying them for years. Emory's screening centers, combined with the ability of our expert chemists to modify compounds, can serve as the glue to enhance the interaction between basic and clinical science and to promote the translation of basic science to the bedside."

"We are not competing with pharmaceutical companies, although some of our findings may well be useful to them on down the road," Dingledine says. "We intentionally are not looking for the next blockbuster antidepressant or antireflux drug. That's not our ball game. We are trying to identify targets that are not being pursued by major pharmaceutical companies yet might have real research value or clinical impact. Academic medical laboratories like ours have a Zen-like focus on understanding the properties of proteins."

### Why Emory got the grant

Emory's history in successful AIDS drug discovery and its track record of collaboration between the medical school and the college's chemistry department gave it a leg up in the selection process for the new NIH screening centers.

"When we went up against some of the biggest-name universities in the country, we were very competitive," says Liotta. "This was because we had ongoing collaborations between chemistry and the School of Medicine that we could point to as real examples of our expertise and our desire to move in that direction. It wasn't a forced interaction—it is what we've done for years.

"Drug discovery is an intimate partnership between biologists and chemists," Liotta continues. "In most places, there is little or no interaction between those two groups, which means that each group is by definition limited in terms of the kinds of questions they can ask and the kinds of answers they can get. Emory's screening center allows for a smooth interface between these two critical groups that enables us to ask and answer the bigger questions. We hope this will make Emory more competitive for external funding and may lead to alternative revenue sources such as drug royalties. Overall, it's an opportunity for us to pool our complementary skill sets and do important work. We hope this also will enable us to recruit the best people, because this extra opportunity is something they don't have at other research institutions."

Former Emory faculty member Jeffrey Conn, now



**Academic scientists know these targets inside out because we've been studying them for years.**

**"If the targets are important and if we discover novel modulators of those targets, then it's our responsibility to file patent applications on them. It's not about money; it's about getting these discoveries into the marketplace for the benefit of patients." —Dennis Liotta**



Chemist Dennis Liotta says drug discovery is an intimate partnership between biologists and chemists.

professor and director of Translational Neuropharmacology at Vanderbilt, heads another of the newly designated NIH Molecular Libraries Screening Centers. "Emory is in an excellent position to play a major leadership role in what may be the early stages of a major shift in biomedical research," said Conn. "Because of its culture of strong collaborations between clinical and basic scientists, I see a translational mindset of scientists across Emory. The Department of Pharmacology has an uncommon combination of strength in classical pharmacology, and the chemistry department is world-class—in fact one of the rare chemistry departments with an established track record in discovery of marketed drugs."

Carson Loomis, program director of the NIH Molecular Screening Libraries Division of Extramural Research at the National Human Genome Research Institute, says Dingleline's leadership in assay-development and high-throughput screening helped Emory capture the molecular libraries designation. "The award was based on the quality of his scientific staff, his development plan, and the proven record of scientific success at Emory," he says.

### Cooperation versus competition

"The MLSCN has a really nice feature that's different from any other NIH award with which I have been involved," says chemistry's Jim Snyder. "There are ten centers around the country, one of which is at the NIH, and this network is meant to be collaborative. We all get on the phone once a month and share information. We try to lower the cost of software and pool ideas about how we can more efficiently do the tasks of molecular screening."

Emory will continue screening proteins identified by its own faculty members in the Emory Chemical-Biology Discovery Center at the same time it screens the NIH-identified proteins. A great deal of the equipment and personnel in the two centers will overlap. Intellectual property issues have required complex negotiations between the NIH and Emory's Office of Technology Transfer. The NIH wants all results of the MLSCN to be transferred quickly into the publicly accessible PubChem database. After much debate, however, it agreed to allow the screening centers and investigators a short time to file patent applications on their discoveries. Compounds not immediately patented will go

into PubChem. Many Emory scientists feel strongly that protecting intellectual property is critical for the future of therapeutic development and for the ultimate good of patients.

"If the targets are important and if we discover novel modulators of those targets, then it's our responsibility to file patent applications on them," says Liotta. "If we don't, and they get into the public domain, no one will ever develop them commercially. It would be terrible if we found something that was potentially an important therapy but no one would spend the money to develop it because we didn't patent it. That's when the public loses. It's not about money; it's about getting these discoveries into the marketplace for the benefit of patients."

"This screening center is going to have a huge impact on tech transfer for a number of reasons," says Todd Sherer, director of technology transfer at Emory. "Venture capitalists and large pharmaceutical companies are not as interested in commercializing early-stage technologies and target proteins as they were five years ago because of changes in patent laws. That means we

have to find ways to move target technology further down the innovation pathway and create better investment opportunities.

"This screening center will help us cross the 'valley of death' that stands between many laboratory research discoveries and getting drugs into the marketplace. This ability to create more compelling intellectual property technology is a crucial part of our ability to be successful in the future."

### A winning combination

Two of the first three proteins selected by the NIH MLSCN for screening at Emory included Fu's 14-3-3 protein and a protein called BAP-1, identified in 1998 by Emory biochemists Keith Wilkinson and Frank Rauscher. BAP-1 interacts with the BRCA1 protein, which is a product of a breast cancer suppressor gene that when mutated promotes the growth of breast cancer. Wilkinson and Rauscher hope they may be able to slow the growth of breast tumor cells by finding a compound to inhibit this protein. After first screening the BAP-1 protein in Emory's library of compounds, they now are screening it through the MLSCN library.

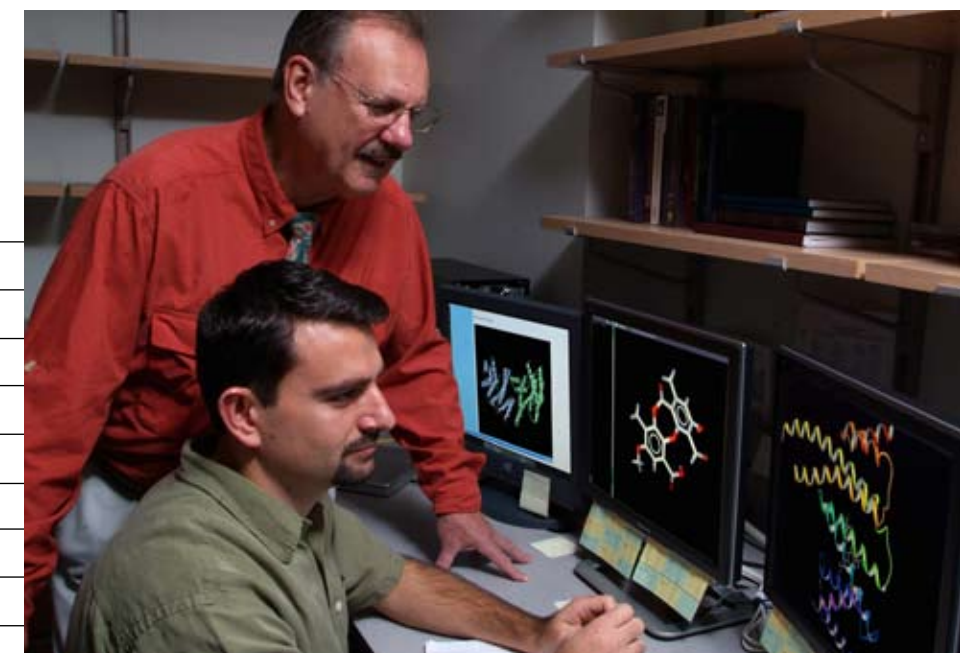
**In addition to the 100,000 additional compounds Emory received through its Molecular Libraries Screening Center Network status, the NIH awarded funds to purchase more screening equipment. The Georgia Research Alliance pledged an extra \$400,000 for equipment, and the three-year NIH grant also has been used to hire additional staff to carry out screening and subsequent chemical modeling and refinements.**

"Before these centers existed, we would have been reduced to trying to talk a drug company into doing this kind of screening," says Wilkinson. "That is difficult if you can't point to the disease and to the nature of a drug that might develop from such a screening. Pharmaceutical companies are not in the business of generating reagents for curious biologists."

Scientists from throughout the School of Medicine

are beginning to use the Emory Chemical-Biology Center to advance their research. Neurologist Jonathan Glass is looking for compounds that might inhibit a protein that causes toxic nerve reactions to the anti-cancer drug taxol. Microbiologists Richard Plemper and Richard Compans, along with Jim Snyder and Dennis Liotta, are trying to develop an antiviral drug for measles. In the first round of high-throughput screening based on a cell fusion assay, they identified more active and promising compounds than they had identified in the past two years working with conventional chemical synthesis. Biochemist David Lambeth is studying a target protein related to the inflammation involved in cardiovascular disease and cancer. Stem cell expert Marie Csete and Dingleline are planning to look for compounds that would reduce the ability of cultured stem cells to differentiate in the laboratory or direct them to differentiate into specific types of cells.

"Emory recognized the opportunity for this kind of high-throughput molecular screening earlier than most



academic institutions and was fortunate to receive the support to move forward," Dingleline says. "Others are now recognizing the advantages of combining chemistry and biology and pharmacology. The national network will move us in exciting new directions, with expertise and interests that by design are complementary rather than competing. No individual center could do all this on its own, but together we can accomplish a great deal."