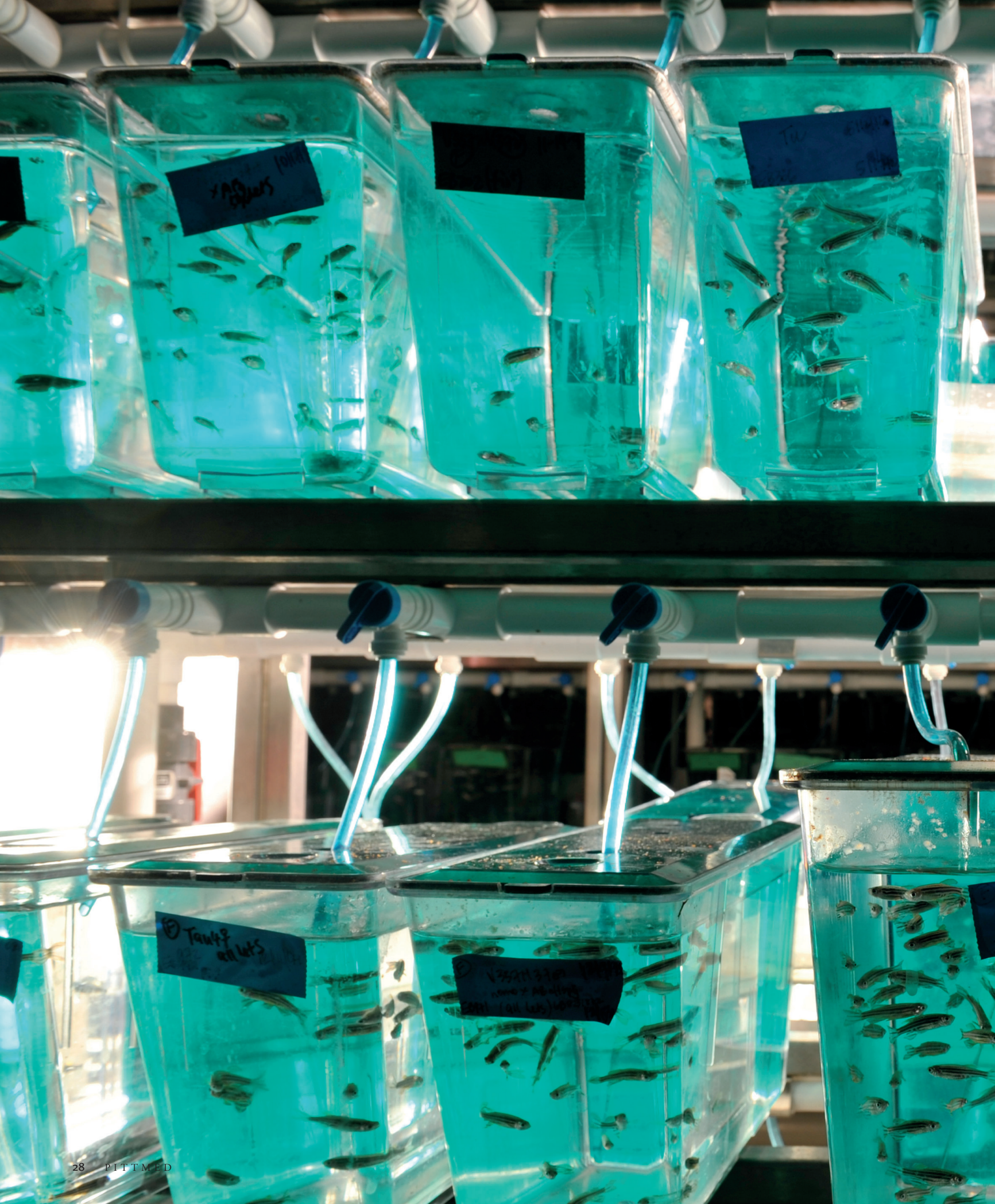


HOW TO NAVIGATE THE MAZE OF DRUG DISCOVERY

BY REID R. FRAZIER

THE LABYRINTH

Pick a disease, any disease. Head and neck cancer? Fine.

Let's say you have a pretty good idea about its major mechanisms. You know one or two of the thousands of proteins inside a cell go "off the tracks," producing cell signals that, unimpeded, will result in a tumor.

How do you stop this from happening?

A logical choice is a drug. Perhaps a pill.

So you look for a chemical compound that will bind to that protein and block the bad signaling pathway from starting up in the first place. It's like slashing the tires of cancer's getaway car before it has left to rob the bank. And this is how many of our drugs work. Statins, the most lucrative drug class in the world, bind to an enzyme that produces cholesterol.

PHOTOGRAPHY | JIM JUDKIS

The Drug Discovery Institute is "embracing the complexity of life, not running from it," with models like zebra fish. By the way, Pitt has up to half a million of these transparent swimmers in its research reservoir.

There are more than 1 billion known chemical compounds, and 25 million of these are kept in vast chemical libraries owned by pharmaceutical companies. About 25 years ago, drug makers began screening millions of these compounds for "hits" on various biological targets—usually enzymes and other proteins critical in the pathogenesis of a disease. Thanks to robots that could fill hundreds of microtiter wells in a few minutes, large-scale testing became the industry standard. This method is called high-throughput screening. The idea is to scan as much of the known chemical universe as quickly as possible to find potential drugs. Many new drugs were found in this way.

High throughput is a breakthrough technology, but it's not perfect. The majority of molecules that record "hits" don't become viable drugs. They may be toxic, or will not necessarily interact in a human the way they will in vitro. So, while our ability to find suitable drug candidates has grown, the "pipeline" of promising drugs in development has, if anything, shrunk in the past few years.

This problem has stuck in the craw of D. Lansing "Lans" Taylor for several decades now. In 1982, Taylor, a cell biologist who was among the first to use digital technology, microscopy, and fluorescence imaging, started up Carnegie Mellon University's Center for Fluorescence Research. A few years later, he began consulting with a handful of pharmaceutical companies. He'd never seen how drug discovery worked. "I was appalled," says Taylor. "I thought the pharmaceutical industry had over-industrialized and simplified the process, reducing the human being to isolated proteins in wells."

Taylor thought that, in their rush to do rapid screens for new drugs, big pharma had missed an obvious, critical point: There's no drug that interacts with only one molecular target. Most interact with many, in ways that scientists are only now beginning to understand.

To Taylor, the answer was to find out what the chemical was doing not just to the particular target protein, but to the whole cell, or a group of cells. In essence, to get more and better information about the drug's effect on the animal up front.

To do this, he thought, drug discovery needed better tools, and he coined the term "high content" screening to describe the process. The concept was to test a drug not just against an isolated, purified protein, but inside a cell, or a whole organism, and report the drug's effect with as much detail and specificity as possible. This took longer than a screen built for speed, but it could help make better decisions about how to order research priorities. Taylor left Carnegie Mellon in 1996 to start a series of biotech companies that used fluorescence to look at drug interactions more deeply. In so doing, he changed the way experimental drugs are investigated.

After a decade and a half away from the academy, Taylor took the helm of Pitt's Drug Discovery Institute in November 2010. Founded in 2006, the institute has been one of about a dozen academic high-throughput screening centers around the country. But in recent years, its researchers in biomedicine, public health, pharmaceutical science, and chemistry have made significant inroads into the kind of high-content areas Taylor has been espousing for a quarter century.

"The old paradigm was one drug, one target, one disease," says Pitt's Ivet Bahar, John K. Vries Professor and chair of computational and systems biology and a Drug Discovery Institute associate director. "You look for a specific drug targeting a specific protein. It's widely understood this paradigm is invalid."

Drug discovery is more complicated than that. It's not a hill to climb, nor a path to follow. It's a labyrinth. Taylor wants to navigate it. He thinks Pitt is the place to do so.

IN THE FISH LAB

At 3:30 on a brisk winter afternoon, it is time to look at the zebra fish. A research assistant in Michael Tsang's developmental biology lab pulls four plastic trays wrapped in heavy duty aluminum foil out of an incubator the size of a mini-fridge. Inside the trays are 96 shallow wells. Each well is filled with day-old zebra fish embryos, each treated with a different chemical compound. The fish have fluorescent tags (green fluorescent protein) from jellyfish embedded in their DNA. The transgenic fish make the fluorescent protein in response to fibroblast growth factor, or FGF. FGF is implicated in a number of important biological pathways, including wound-healing and cancer. Tsang is looking for a drug that will ramp up FGF to aid with healing in heart patients. His test: Wherever an embryo expresses FGF, its will fluoresce under UV light.

Zebra fish are a valuable model. For starters, their eggs are fertilized outside of the mother, enabling researchers to take control of embryos a few hours old. They share 70 to 80 percent of their genetic makeup with humans, and they develop many organs within 24 hours of fertilization. So Tsang can do a high-content drug screen on the embryos in a reasonable amount of time. (Another similarly elegant and helpful model is *C. elegans*, the tiny nematode. It's helping Pitt pediatric researchers look at ways cells clean up toxic proteins in an effort to prevent cellular necrosis.)

A few years ago, Tsang's screening of zebra fish showed a chemical called BCI increased FGF activity. Since BCI proved to be toxic at higher levels, he's been searching for other FGF promoters. So he's been putting hundreds of chemicals into the wells every week, looking for some light. On this afternoon, Tsang's research assistant, Manush Saydmohammed, takes the trays up a few floors to a room inside the Drug Discovery Institute in Pitt's Biomedical Science Tower 3. The trays go inside a fluorescence laser scanner, and a few minutes later, the first zebra fish embryo appears in black and white on a nearby monitor. The embryo consists of a yolk and the beginnings of a fish body starting to grow around it. Magnified, it looks like a shrimp trying to crawl on top of a pea. A pair of bright white areas show up on the scan: one at the larva's eye, another a little further back, toward the dorsal area. This is the fish's mid-hind brain. In both areas, the fish is expressing FGF.

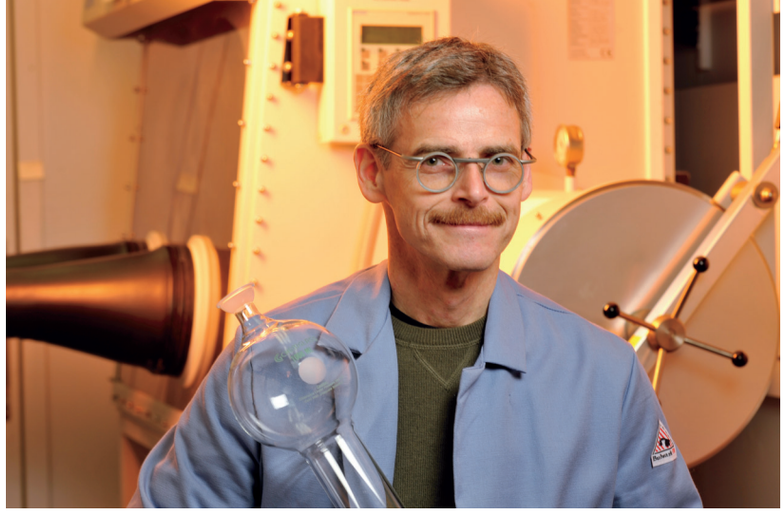
The scanner records images from all 96 embryos. Each image will be analyzed by an algorithm Tsang and his collaborators designed to quantify FGF expression. In some of the wells, the embryo is nothing more than a gray shadow, a likely sign that the compound was toxic. In one of the screens, a larva has spots as bright as those of its BCI-laced brethren.

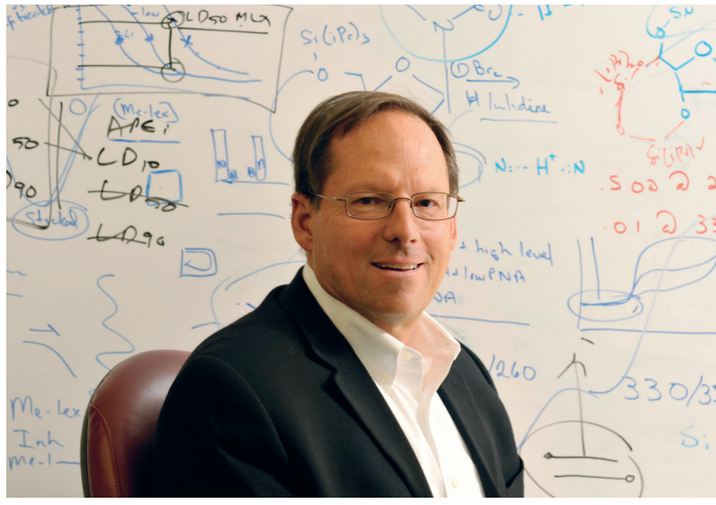
"That's positive," Saydmohammed says.

When Tsang pops into the room a few minutes later, Saydmohammed tells him, "We found one that looks like a hit."

"As much as BCI?" Tsang asks. Tsang has a look of pleasant surprise. It could be promising, but Tsang knows that the experiment will have to be duplicated. If, indeed, it is a potent FGF promoter, a whole new chapter in the work of creating a drug begins.







The University of Pittsburgh's Drug Discovery Institute leadership team includes D. Lansing Taylor, its director (in red tie), and associate directors (clockwise from left) Ivet Bahar, Peter Wipf, Barry Gold, and Edward Chu (not shown).

THE TRIAL AND ERROR OF OUR WAYS

Of an estimated 320,000 plant species on Earth, perhaps as many as 25,000 have been used for some type of folk medicine. Plants were a mainstay of drug discovery for much of human history, usually involving a process of trial and error. Scientists began isolating different plant and mineral chemicals, but finding ones that are safe in humans was remarkably difficult. Only about 120 plant-derived chemicals are in use around the world today.

Plants produce many of their chemicals as toxins to ward off predators. Minerals, another source of early medicine, are seldom able to penetrate the body's biological membrane barriers. Those that do are often highly toxic. Modern imaging, chemistry, and genetics have made drug discovery—whether through plant- and mineral-derived or synthetic compounds—much, much faster, but the process remains riddled with pitfalls.

To be both effective and safe, a molecule must be somewhat water soluble, have suitable places for hydrogen bonds to form, and be small enough and the right shape to "squeeze" into the archipelago of structures on a protein. If a compound isn't metabolically stable, the body will degrade it before it has a chance to reach its cellular target.

"You're talking about many years of optimization, follow-up assays, animal models," says Pitt's Peter Wipf, a Distinguished University Professor of Chemistry with a joint appointment in pharmaceutical sciences and chemistry. "You have to study pharmacodynamics, pharmacokinetics, toxicity, metabolism, absorption, solubility, formulation," before you can think about putting it into humans.

"It is an extremely complex pipeline," says Wipf, who is director of Pitt's Combinatorial Chemistry Center, as well as an associate director of its Drug Discovery Institute. "In fact, it's not really a pipeline. It's more like a maze of different possibilities."

The time between when a compound produces a "hit" on a large chemical screen and when it gets tried in humans is what Barry Gold, professor and chair of pharmaceutical sciences, affectionately calls "the Valley of Death." Gold is a Drug Discovery Institute associate director along with Wipf, Bahar, and Edward Chu, a professor of medicine and deputy director of UPCI.

"Chances of success," Gold admits, "are low. We can't really predict toxicity accurately. Small changes to a molecule can have major changes to its activities." A small methyl group, for instance, can alter the shape of a molecule and, therefore, its activity.

Advanced computational analysis of DNA, molecular and protein structure, and polarity help rule out some types of compounds, but most must be tested either in vitro or in vivo.

Ongoing research in Gold's academic home, the School of Pharmacy, focuses on metabolic stability, predictive models for protein binding, and speeding up ways to synthesize drug compounds. All of these are ways of identifying compounds that may be toxic.

"It's important as early as possible to get that type of information, because you really don't want to be developing a molecule that's going to be toxic down the road in animals," says Gold, whose lab focuses on an inhibitor of protein interactions critical in cancer drug resistance.

Even with the rise of genomics and our new understandings of how proteins work, drug discovery involves one element no scientist likes to rely on: luck. Take the platinum-based cancer drug cisplatin. The biophysicist who found it wasn't looking for a cancer drug. He was studying DNA synthesis in *E. coli* when he observed the bacteria weren't dividing when exposed to a platinum electrode.

"Cisplatin was discovered by pure accident," says Gold. "Someone discovered trace amounts of cisplatinum would inhibit DNA synthesis and induce toxicity. It just came out of the blue."

The researcher who discovered cisplatin may not have been looking for an antitumor agent, but he knew what to do with it once he found it. "In a way, drug discovery is a lot like fishing," says Gold. "A lot of it is luck. But the key in fishing is: Don't fish in vacant waters."

Ivet Bahar is exploring what she believes are bountiful waters.

Bahar is one of the world's leading thinkers in protein biomechanics. Her lab has gained renown for creating computer models for protein movement. Parts of proteins, called domains, can act like spring-loaded door hinges. During cell signaling, they can swing open or slam shut. Each position alters the activities of the protein. In the digital movies she and her collaborators have made to model protein movement, proteins look like balls of yarn and ribbon, bobbing up and down underwater. The models help predict a protein's "druggable sites" for which a chemist can design a compound.

On a recent afternoon, Bahar leaned over a laptop. A video on the screen showed a 3-D, computer-generated form composed of blue and white spheres. It looked more or less like a cloud as seen from an airplane.

"What you see, like a cloud, is the protein, the surface of the protein," says Bahar. The spheres are parts of the protein, colored according to hydrophobicity, polarity, and other chemical characteristics. A series of geometrical shapes jump around the blue and white protein quickly, kind of like Mexican jumping beans. These are small molecules interacting with various sites on the protein.

Among the proteins of interest to Bahar's lab is DUSP6, which Tsang is trying to block because it inhibits FGF expression. Bahar's lab is helping Tsang model the protein and find out what molecules are most likely to interact with it.

When he analyzed a crystallography image of Tsang's protein, Bahar's postdoctoral fellow, Ahmet Bakan, created a dynamic model of the protein. The model revealed that Tsang's FGF-promoter and drug candidate, BCI, made for a pretty weak binder. The model predicted another site where a much tighter bind could occur. Tight binds are preferable, because they increase the chances that a compound will only bind to that site, rather than other, "off-target" sites. This results in fewer side effects.

Bahar says her group can predict internal motion in a protein in the same way one can predict how a door hinge or human elbow moves. "These are motions that the structure favors. Its structure has evolved to favor particular motions that are needed for its function," she says. "This is evolutionary selection."

BUILDING A (DRUG) SEARCH ENGINE

Taylor arrives at Pitt as the world beneath drug discovery is shifting. Big pharma's drug pipelines are thinning, and the industry faces a "patent cliff" as many of its blockbusters are soon to go off patent. Consequently, the industry as a whole has started to scale back, laying hundreds of thousands of employees off around the world. National Institutes of Health funding isn't likely to increase for research that feeds drug discovery anytime soon, Taylor reckons. He sees partnerships between academia and the private sector as necessary in drug discovery.

He is quick to point out there are caveats—the University needs to maintain academic autonomy, avoid conflicts of interest, and recoup an equitable share of any proceeds from drugs developed in its labs. "I think it's important we not just become the front end of a pharmaceutical company," he says.

Each side, he says, stands to gain from such an arrangement. Industry can gain expertise in basic biological sciences. Academia can gain access to the industry's vast chemical libraries, equipment, and money.

Taylor's own experience gives him a unique vantage point from which to view the academic-industry divide. Taylor says he caught "the entrepreneurial bug" while at Carnegie Mellon. In 1996, he left that university to start a biotech company called Cellomics. The company made imaging equipment, imaging software, information management software, and reagent kits for the high-content screening Taylor thought crucial to drug discovery.

This was during the height of the push for high-throughput screening, and Taylor's ideas were met with doubt. "Always in the front row, somebody from the screening facility would say, 'What's the throughput? How long does it take to read?'" The first generation of his screening tools was slow. He told the lab folks it would take 25 minutes to measure a 96-well plate. A high-throughput system took less than a minute. People walked out in the middle of his presentations, Taylor recalls.

But he persisted, and Cellomics was eventually successful enough that it was acquired by Thermo Fisher Scientific.

"I always thought he was a visionary," says Alan Waggoner, whom Taylor recruited to Carnegie Mellon to start its fluorescence research center. "He could see things that could be done with fluorescence detection way ahead of a lot of people—sometimes before it was really practical." (Taylor and Waggoner started Biological Detection Systems in the early 1990s; it is now part of GE Healthcare.)

Taylor's ideas about fluorescence originated when he was a grad student in the 1970s under the famed microscopist Robert Allen at the State University of New York–Albany. Taylor was interested in cell movement. He thought fluorescence could help study this. He stuck fluorescent dyes on proteins involved in movement and watched them under ultra-low light conditions.

"The first image (created in 1974) was from a recently declassified night-vision camera I got from the military," Taylor says. He also began linking cameras to computers, allowing his lab to quantify what the camera saw.

Taylor went on to found a total of three companies based on high-content concepts. His career as an entrepreneur was rewarding, yet he decided to re-enter the academic world last year.

"I want to take some risks here and do some things in academia that pharmaceutical [companies] can't afford to do now," he says. "We're taking this concept of complexity a step further. We're embracing the complexity of life, not running from it."

"I think everyone realizes what we need to do must have practical value and not be just a bunch of neat science," notes Gold. "That's what we're trying to do—to get a drug out." ■