

The Chemical Biologists

Small molecules and “systematic serendipity” yield big ideas in life-sciences and biomedical research.

by Patricia Thomas

ALTHOUGH STUART L. SCHREIBER'S office number is 223 Conant, one of several buildings in the chemistry complex, only part of his suite is actually within its walls. When he steps outside his art-filled private office to talk to a graduate student or grab a file, he crosses an invisible line into the Fairchild Biochemistry Building, which is packed with life-sciences labs.

Having an office that physically spans these two worlds is metaphorically perfect for Schreiber, Loeb professor of chemical biology and one of several Harvard scientists who have been chipping at the wall between chemistry and biology to make way for an interdisciplinary enterprise called chemical biology. One of Schreiber's many contributions has been in helping to develop technology now used by nearly all chemical biologists: robotic equipment that rapidly screens thousands of “small molecules” to see if they perturb a specific biological activity. Small

molecules are chemical compounds with a molecular weight of 500 Daltons or less—about one-fiftieth to one-hundredth the size of most proteins. Yet when a small molecule latches onto a receptive protein, the protein's shape is changed in a way that can make it more—or less—able to carry out its mission in the cell.

Scientists look for these hookups between chemicals and proteins using “high-throughput” screens that test 384 small molecules at once in a plastic plate not much bigger than a deck of cards. Some experiments seek compounds to halt the proliferation of cancer cells; others look for molecules that can kill drug-resistant forms of staphylococcus or *Mycobacterium tuberculosis*. Sometimes the screens pick out small molecules that do these things but are restricted to laboratory use due to safety issues

that make them unsuitable as medicines. Scientists are optimistic, however, that a few of these newly discovered compounds can become tomorrow's most important drugs—not just billion-dollar blockbusters in wealthy countries but long-sought cures for global scourges like malaria, TB, and AIDS. In research laboratories, the utility of small molecules is bound only by human imagination. The enormous promise that chemical biology holds for medicine—and fundamental discovery—has made it one of the most exciting fields in the life sciences.

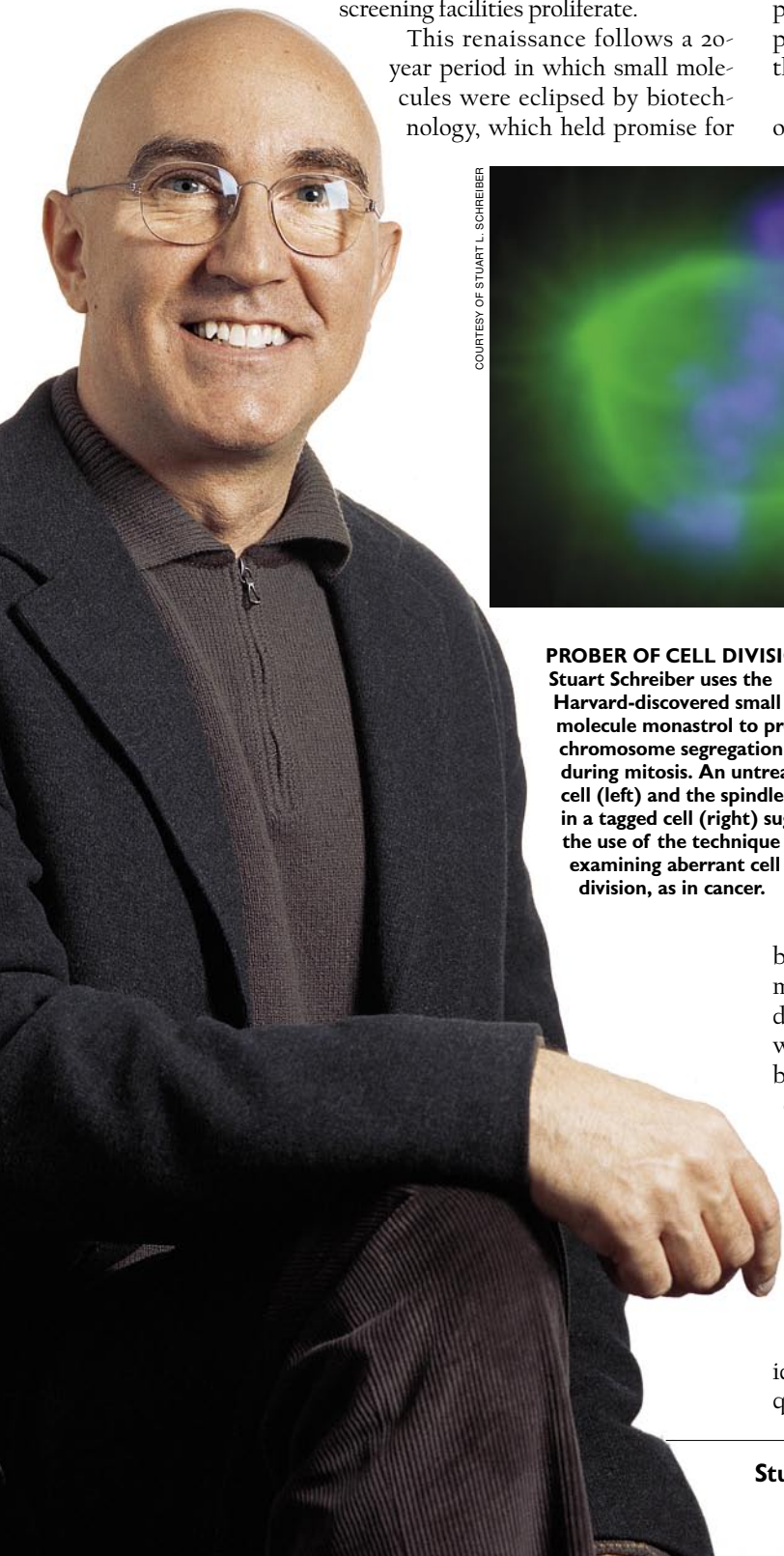
Last fall, the University gave this new

discipline a boost by launching the nation's first Ph.D. program in chemical biology, codirected by Jon Clardy, professor of biological chemistry and molecular pharmacology at Harvard Medical School (HMS), and Gregory L. Verdine, Erving professor of chemistry and Harvard College Professor in the Faculty of Arts and Sciences (FAS). At the same time, an introductory course on chemical biology—Science B-47, called “Molecules of Life”—made its debut as part of the undergraduate Core curriculum. Since then, the field has been evolving faster than Darwin’s finches as additional faculty members are recruited, new small molecules are created, and high-speed screening facilities proliferate.

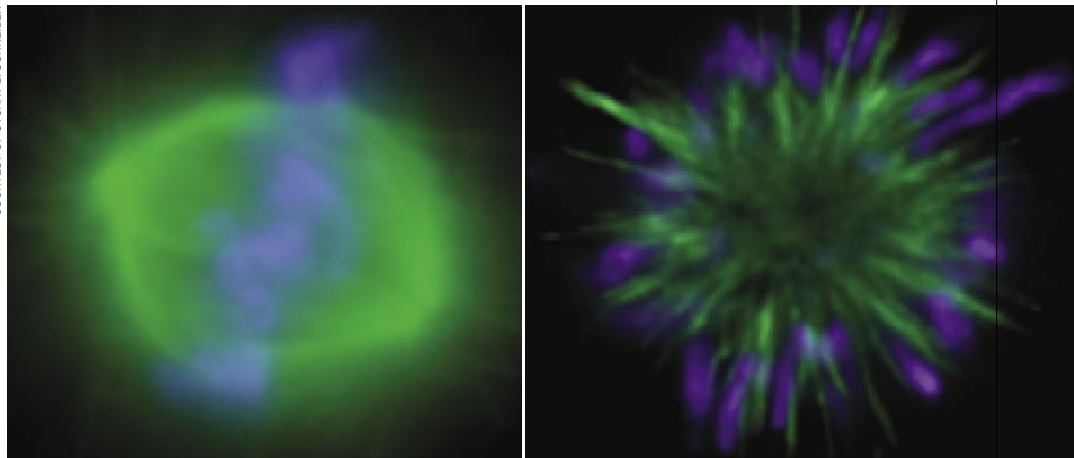
This renaissance follows a 20-year period in which small molecules were eclipsed by biotechnology, which held promise for

developing large molecules—either proteins or parts of proteins called peptides—as medicines. When biotechnology exploded in the early 1980s, it seemed wildly exciting compared with medicinal chemistry, the 200-year-old science of making small-molecule drugs. Some scientists thought that small-molecule chemistry had accomplished all it could in the battle against disease. Today, in the post-genome era, almost no one would agree with that dour appraisal of small molecules: having complete genome sequences makes it theoretically possible to generate “proteomes,” comprehensive sets of all the proteins that constitute a human being or a microbe that makes people sick. And disease-producing proteins, whether they clog the brains of Alzheimer’s patients or enable HIV to penetrate a human cell, may be thwarted by small-molecule drugs.

Hundreds of thousands of small molecules course continuously through the human body. Key players like steroids, neuro-



COURTESY OF STUART L. SCHREIBER



PROBER OF CELL DIVISION: Stuart Schreiber uses the Harvard-discovered small molecule monastrol to probe chromosome segregation during mitosis. An untreated cell (left) and the spindles in a tagged cell (right) suggest the use of the technique in examining aberrant cell division, as in cancer.

transmitters, pheromones, and prostaglandins are essential for maintaining health, fighting illness, and mounting a fast and fitting response to a kiss or a car crash. The world outside the body teems with millions more small molecules, some of which have proved to be “winners in human medicine,” says Christopher T. Walsh, Kuhn professor of biological chemistry and molecular pharmacology at HMS.

Penicillin, cyclosporine, and hundreds of other small molecules that microorganisms wield for their own benefit have been copied—and altered—by human chemists to make them into valuable medicines for people. “Before 1980, all drugs were small molecules, with a few exceptions like insulin, which is a small therapeutic protein,” says Walsh. “Aspirin, Celebrex, Prozac, Lipitor—nearly every familiar drug is a small molecule.” These compounds are desirable medicines because they can be taken as pills: their diminutive size enables them to pass easily from the gut to the bloodstream, which swiftly transports them to where they’re needed to relieve a headache, fight strep throat, or relax a knotted muscle. Bigger molecules must be injected (they can’t pass through the digestive system into the bloodstream) and, when given the choice, nearly everyone prefers pills.

The current small-molecule revival has chemical biologists identifying and synthesizing novel molecules, framing biological questions that small molecules might help answer by blocking or

activating protein targets, and screening molecules against these targets to look for bull's-eyes. Some labs focus on one of these tasks; others do all three. One leading practitioner describes chemical biology at Harvard as "a big tent." A look under the big top reveals a glittering array of biologically oriented chemists and chemistry-savvy biologists developing new paths in fundamental research and exploring the far reaches of small-molecule medicine.

"Natural-products" chemistry and chemical synthesis are two classic approaches to making small molecules. HMS investigators Walsh and Clardy are nationally recognized leaders in natural-products research; Harvard's best known synthetic chemist is Schreiber. In recent years, these traditional strategies have been joined by new ones that harness the power of natural selection to create entirely new compounds, or use structural wizardry to turn a floppy chemical structure into a fierce cancer fighter by stiffening its spine.

Prospecting for drugs

THE FIRST STEP in a typical Jon Clardy experiment is not *making* small molecules, but foraging for them. Armed with a trowel, Clardy spent the early years of his career grubbing for microbial DNA in rain-forest soil and wading in the shallows of the Sea of Cortez in Mexico, scraping sponges, algae, and crustaceans off rocks. During one especially productive period, he and his colleagues determined the chemical structures of about 40 previously unknown compounds, including at least two famous ones. Saxatoxin can poison marine life and people swept up in a red tide; bryostatin, isolated by Clardy from a fungus he found on wharf pilings, stops the growth of certain cancer cells. Some of modern medicine's most powerful antibiotics and immune suppressants come from such soil and marine microbes, most of them difficult or impossible to grow in the laboratory.

But Clardy has little time for "front-end collecting" these days, given his responsibilities as codirector of the new chemical biology doctoral program, lab chief, and teacher ("Molecules of Life" is one of his courses). Fortunately, members of his research team are adept at gathering DNA samples in the wild, isolating and inserting microbial genes into docile workhorses like *E. coli*, and ultimately harvesting an assortment of biologically active small molecules. With such accomplished colleagues, Clardy jokes, "I'm like a lawyer—I only see people when they're in trouble." In fact, he remains the go-to guy when someone is stymied by the structure of a new molecule.

Because Clardy is an academic researcher, he can pursue inquiries that would not get far in a drug-company lab. Lack of commercial potential, he says, forces corporate researchers to discard many interesting, bioactive small molecules that academic researchers are happy to use as bioprobes for investigating how cells communicate, proliferate, or die. And when academic researchers study molecules as possible medicines, they don't have to be obsessed with the bottom line. "Companies are working to find the next Viagra or Prozac," says Clardy, "but they're not working very hard on antibiotics."

Academic researchers, in contrast, are able to tackle some of the world's leading causes of death, whether or not the people affected can afford to buy medicines. For example, Clardy and scientists from the Harvard School of Public Health plan to pit

some of his lab's small molecules against possible weak spots in malaria- and tuberculosis-causing organisms. "People with chemistry and small-molecule expertise and high-throughput screening equipment have great synergy with experts on pathogens and potential drug targets," Clardy says. "Few other places can put together this kind of combination."

Playing chess with Mother Nature

LIKE CLARDY, Christopher Walsh is a specialist in natural products. He is fascinated by the inner logic that microorganisms use to formulate the small molecules essential for defending themselves in a bug-eat-bug world. We know these substances as antibiotics: invaluable medicines that attack the cells of prokaryotic bacteria or fungi without damaging eukaryotic human cells.

"All medicines are molecules made by chemists, either natural or human



Schreiber's new vision is that chemical biologists should compile a library of roughly 500,000 small molecules that could block or activate every protein in the human body.

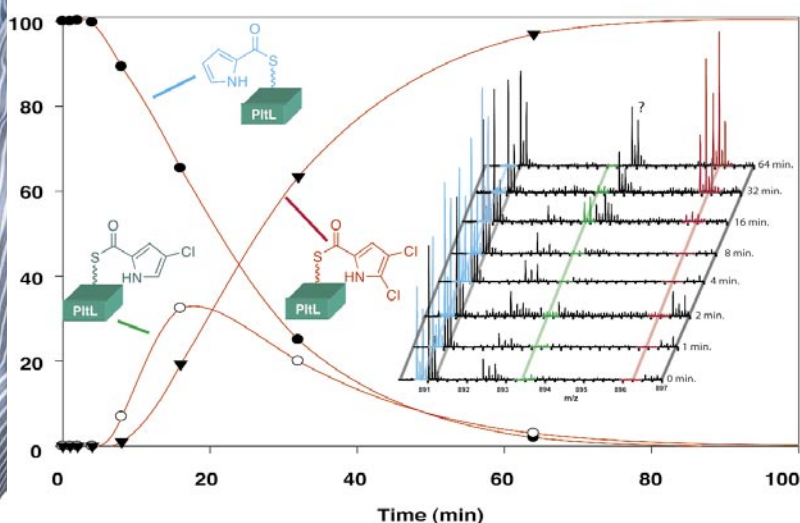
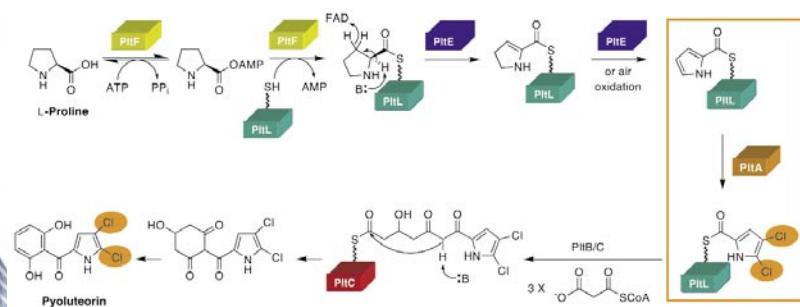
chemists," says Walsh. His lab has published a series of discoveries about microbial assembly lines that churn out polyketide antibiotics, such as erythromycin or tetracycline, and polypeptide antibiotics like vancomycin. Walsh and his colleagues have identified various work stations on these assembly lines and are now reprogramming enzymes at these stations to synthesize souped-up models of compounds they've made for millennia.

The sequencing of about 150 bacterial genomes has accelerated this work. Having genomes for prolific natural chemists like *Streptomyces* and *Actinomyces* helps scientists create a "parts list" of enzymatic proteins made by each organism, "and the more extensive the parts list, the quicker you can build something and the more variation you can try," Walsh explains. Genomic data also make it easier to spot cracks in a pathogen's armor. "Genome information took infectious disease from a target-poor to a target-rich therapeutic area," he adds, "and if you

have new targets, you have an opportunity to make new drugs."

Infectious diseases are a leading cause of death worldwide: even in the best U.S. hospitals, many patients succumb to drug-resistant infections. Walsh believes the waning interest of pharmaceutical companies in antibiotics and antiviral drugs leaves "a vacuum that can be filled by top academic medical research centers: to create new knowledge, help small companies start, and provide the intellectual base for companies that have stayed in the field." (One thing the University should not do, in his opinion, is strive to become a pharmaceutical or biotech company: "We don't make drugs for a living. We're amateurs." But by serving as a scientific adviser to companies large and small, he acts as "the translator, ensuring diffusion of new knowledge into new technology. My role there is to teach people new principles." In this way, what happens in the academy shapes the future of drug discovery in the private sector.)

COURTESY OF CHRISTOPHER WALSH AND SYLVIE GARNEAU



BIOSYNTHESIST: Christopher Walsh, who specializes in "natural-products" chemistry, is publishing discoveries on the biosynthesis of pyoluteorin, an antifungal agent (top), and of chlorination reactions involved in reconstituting that assembly process (the bottom time-course diagram).

When more is better

CHEMICAL BIOLOGISTS like Walsh and Clardy start with clues from nature about what might be needed to accomplish a specific task, such as killing drug-resistant *Staphylococcus*, and work toward this goal by elaborating on nature's original idea. Stuart Schreiber operates on a different scale. Over the years, his lab has generated many thousands of new compounds that may be able to bind protein targets, changing their shape and how they function in cells. His new vision is that chemical biologists should compile a library of roughly 500,000 small molecules that could selectively block or activate every protein in the human body. Sequencing the human genome took about a dozen years; Schreiber estimates his plan could be complete in a decade.

In theory, this "perturbogen" library would enable researchers to analyze what individual proteins are doing as organisms develop and interact with the world around them. As it is, biomedical researchers sometimes feel like a befuddled householder in a dark cellar, randomly flipping circuit breakers in an unlabeled electrical box, trying to figure out which one activates the refrigerator. The right set of small-molecule probes could slap labels on the switches and turn on all the lights—if that were the goal—pronto.

Schreiber was teaching at Yale when he first became interested in making small molecules to bind natural proteins; his initial success was synthesizing pheromones that cockroaches mistook for the real thing. He soon abandoned cockroach mating signals for molecules with more relevance to human health, and in 1988 he returned to the

Some of these chemicals have a lot in common. Says Liu, “It may be love at first sight.”

Harvard chemistry department, where he had earned his Ph.D. seven years earlier; today he holds an endowed chair and is a Howard Hughes Medical Investigator as well.

During the 1990s, Schreiber was instrumental in advancing two of chemical biology’s core technologies: diversity-oriented synthesis (DOS) of small molecules and high-throughput assays to test their biological function. He refined split-and-pool synthesis, a fairly standard technique, to create libraries by combining small chemical building blocks in every spatial orientation so that all possible new molecules are formed. To create a typical “library” of 10,000 molecules, three to four chemical reactions are used, with each step incorporating approximately 10 chemical building blocks. (For ease of handling, these chemicals are attached to tiny, inert plastic beads about 500 to 600 microns in diameter.) After each chemical reaction, all the molecules are pooled and randomly split so that every chemical reaction and every combination of building blocks is realized. The resulting library of new molecules is contained on about 50,000 beads—enough to fill a shot glass—and a robotic system cleaves the new compounds from the beads and separates them so they can be stored for future screening.

The beauty of this procedure is that it generates an enormous diversity of compounds after only a few cycles. Not all will be useful, however, and scientists may find themselves searching for a needle in a haystack “that is unnecessarily complex, or you may have made a haystack that doesn’t even have a needle,” says Schreiber. A second problem is that DOS may yield interesting molecules in amounts too minuscule for experimental use. Yet even though Schreiber will never be an artisanal drug maker like Clardy or Walsh, he has consciously shifted gears to make chemical libraries that feature fewer molecules in larger quantities. By applying different chemical principles, employing computational models, and using biological screening data to guide synthesis, Schreiber says he can “improve the chances of finding a really interesting needle.”

Harnessing the power of DNA

DAVID R. LIU, professor of chemistry and chemical biology, shares his colleagues’ goal of synthesizing new compounds for use in the laboratory and clinic. His approach to making small molecules, however, blends biology and chemistry in a unique way. The four nucleic acids that constitute DNA match up in a pattern called “Watson-Crick base pairing”—adenine binds

only with thymine and guanine only with cytosine. The powerful attraction these partners feel for one another causes complementary strands of DNA to stick together like zippers, while unmatched ones go their separate ways.

In a process he dubs “DNA-templated synthesis,” Liu selects thousands of organic chemical fragments and attaches them to short strands of DNA. These DNA-labeled building blocks are then combined with a set of DNA templates, longer strands that grab the short pieces of DNA attached to the chemicals. Some of these chemicals would never cross paths in nature, and their affinity for one another would not be strong enough to pull them together in split-and-pool synthesis, where so much goes on at once.

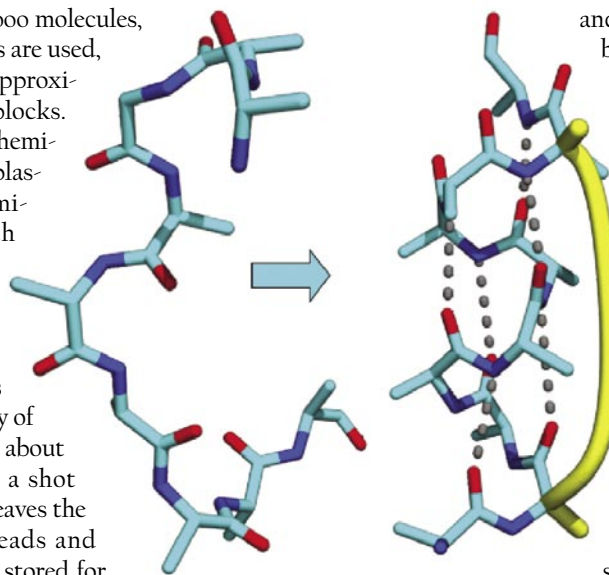
This changes when Liu puts thousands of them into a crucible “the size of a raindrop” and the magnetism of complementary base pairing kicks in. Shoved together like strangers in a subway car during rush hour, some of these DNA-tagged chemicals will discover they have a lot in common. “Once they get together they are very reactive,” says Liu. “They become permanent friends. It may be love at first sight.”

Although this happens on a scale about one-millionth that of conventional split-and-pool synthesis, Liu and his colleagues have no trouble determining which chemicals hooked up because each building block is wearing a unique DNA nametag. These DNA labels also make it easier to screen many compounds at once for their biological properties. “In one experiment, we pass a solution carrying the whole library over an immobilized protein target. The ones with the desired activity stick to the target, while the ones without that action wash away,” Liu says. “Because we

wrote the genetic code for the DNA labels, reading the sequence tells us which ones have the desired property.”

The next experimental step, which he and his team haven’t yet reached, is to put molecules with desirable characteristics through additional rounds of DNA-templated synthesis, mutating their DNA slightly and letting natural selection pick the best new variants. “The point of evolution,” Liu says, “is to find the best compound with the best properties.”

In recent articles in *Science* and *Nature*, Liu and his coworkers—he supervises a lab of 15 to 20 people (and teaches organic chemistry and chemical biology in the College)—report that a single round of DNA-templated synthesis yields new molecules “of reasonable complexity and diversity.” Accomplishments like these have thrust the 31-year-old scientist into the spotlight: *Popular Science* named him one of the 10 most brilliant scientists in the



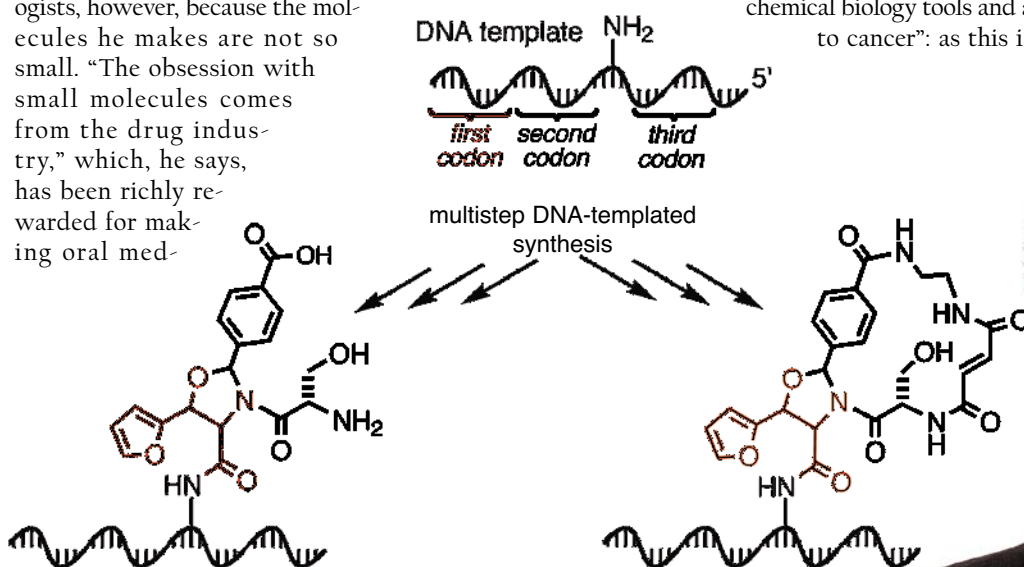
MOLECULAR SCULPTOR:

Floppy peptides—chains of amino acids (left)—become biologically active when folded into a precise structure that meshes with a target, such as a protein. Gregory Verdine constructed a hydrocarbon chain to “staple” a peptide chain into a rigid alpha helix (right). That configuration has triggered cell death in experiments on leukemia.

United States and *Technology Review* included him in its annual list of the world's "top 100" young innovators. Despite such kudos, Liu says he is in the "early to middle stages" of this work: "My hope is that within the next five to 10 years we will be able to discover molecules with useful properties for medicine and other industries."

Not such a small world?

"WHEN YOU'RE AT DANA FARBER [Cancer Institute, in Boston] and you're on the elevator with someone attached to an IV line, it really brings you down to earth," says Gregory L. Verdine. "If there's any way your training and insights can impact human disease, you want to do it." His approach to finding new medicines is different from that of most other chemical biologists, however, because the molecules he makes are not so small. "The obsession with small molecules comes from the drug industry," which, he says, has been richly rewarded for making oral med-



ications. Unfortunately, he adds, the "dirty secret" of chemical biology is that "small molecules bind directly to only about 20 percent of proteins and in many cases the binding isn't very tight."

His laboratory fabricates "intermediate molecules" with molecular weights ranging from 1,000 to 15,000 Daltons, compounds that mimic part of a natural protein and may be able to bind disease-related proteins that a typical small molecule—weighing 500 Daltons or less—cannot. Not surprisingly, Verdine believes that the unifying theme of chemical biology is not the size of the molecules, but the drive to synthesize new compounds.

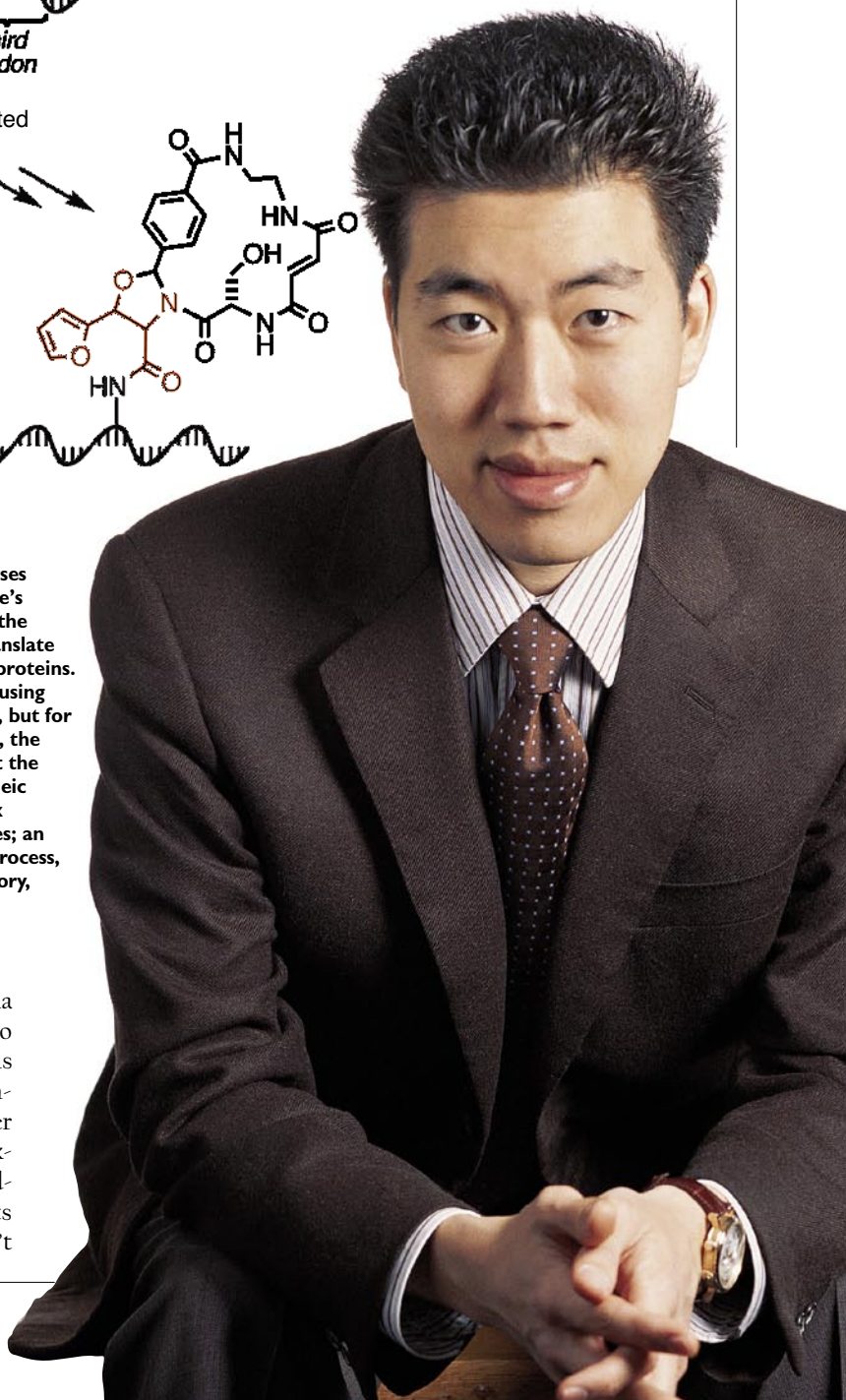
One of Verdine's favorite intermediate molecules is the alpha helix, a sort of trailer hitch that many proteins use to lock onto one another. The first helical structure approved as a drug is Fuzeon, a very expensive, last-ditch treatment for patients infected with strains of HIV that have become resistant to other antiviral drugs. Fuzeon is a floppy helix that fits into a lock-like protein on the surface of HIV like a rubber key, and hardens into the classic alpha conformation only after touching its target. This delay, Verdine says, means that Fuzeon "doesn't

bind as tightly as one would like because it is floppy, not rigid."

Reasoning that it would be better to start with a hard key, Verdine and his team used chemical building blocks to construct a stiffer structure that appears to be a promising weapon not against HIV, but against leukemia. They used a short hydrocarbon chain to "staple" an alpha helix into a rigid form that passes easily through cell membranes and binds a regulatory protein in leukemia cells. This synthetic version of a natural helix locks into place and triggers apoptosis, or programmed cell death, in a mouse model of human leukemia. In a recent issue of *Science*, Verdine and his coworkers report that counts of leukemia cells fell dramatically and tumors regressed in treated animals. "Although this may not look like a drug," he says, "it behaves like one." He has joined forces with researchers at Dana-Farber to "take some of these chemical biology tools and approaches and apply them directly to cancer": as this issue went to press, the Dana Far-

DNA DESIGNER: David Liu's syntheses make use of nature's template. Within the cell, ribosomes translate nucleic acids into proteins. In the laboratory, using DNA as a scaffold, but for different purposes, the scientist can direct the translation of nucleic acids into complex synthetic molecules; an iteration of that process, from Liu's laboratory, is shown here.

David R. Liu



ber/Harvard Program in Cancer Chemical Biology—a collaboration among the Dana Farber, FAS, and HMS aimed at developing entirely new strategies for the treatment of cancer using synthetic molecules—was announced; Verdine is its director.

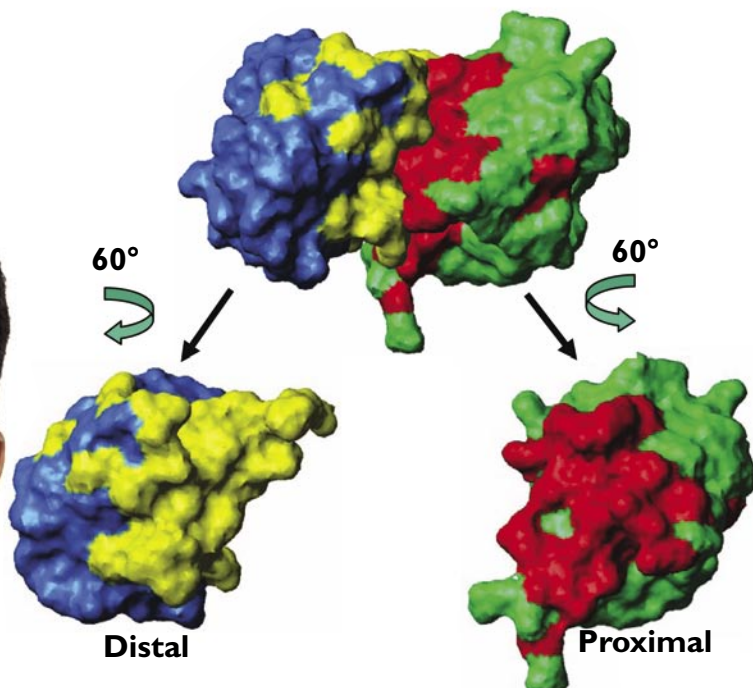
Pioneering “systematic serendipity”

SCIENTISTS WHO WORK with a limited repertoire of small molecules can often sort through them in their own laboratories. But researchers who need to screen vast chemical libraries, searching for the proverbial needle, can't individually afford the equipment and expertise required to work at this scale.

In 1997, well before most universities recognized that faculty members needed help with small-molecule screening, HMS and FAS announced the birth of the Institute for Chemistry and Cell Biology (ICCB), the brainchild of Schreiber, HMS's then-chairman of cell biology Marc Kirschner (now chairman of systems biology; see page 67), and Timothy Mitchison, who was then new to HMS and is now Sabbagh professor of cell biology. Funded by the University, the National Cancer Institute, and Merck, “ICCB was the place where what it means to do chemical biology was defined,” says Jon Clardy. “Other places were doing chemical biology, of course, but

this was the most visible and systematic and large-scale effort.”

ICCB's founders wanted it to be a magnet for scientists with a broad spectrum of interests. “Whatever system you wished to study—aging, memory, cognition, consciousness—we wanted to put in place a set of technologies that would enable you to do so,” says Schreiber, who codirects ICCB with Mitchison. The strategy has worked: the ICCB has facilitated screening projects for more than 80 research groups from Harvard, other American universities, and international institutions.



SANDWICH MAKER: In another demonstration of the importance of molecular shape, Randall King explores the enzymatic attachment of ubiquitin, a small protein, to other proteins, altering their function or subjecting them to cellular degradation. Here, two ubiquitin molecules (blue and green) join together where bound (areas in yellow and red) by another compound under investigation, ubistatin, which acts “like the mayo on a sandwich.” Ubistatin has the interesting property of thwarting the cell's natural self-cleaning function.

Scientists typically come to the ICCB because they've hit a wall with manual assays and need technical assistance and fast, automated machines to proceed with high-volume experiments. Users are required to provide their own experimental materials and personnel, and to share data about the molecules they test. ICCB staff members provide technical advice and help run the screens. After investigators have published their results, experimental findings are entered into a database called ChemBank, fleshing out profiles that will help future researchers select small molecules suitable for their own experiments.

ICCB's appeal is not aesthetic: it occupies a large utilitarian room just off the HMS Quadrangle, with bland linoleum floors and many gray metal shelves. Prominent

Randall King

features include a wall of giant brushed-aluminum freezers packed with small-molecule libraries, lab benches where visiting investigators can culture materials needed for assays, and a motley collection of automated machines and computers.

The heart of the operation is a three-inch-by-five-inch plastic plate, about one-half-inch thick and pockmarked with 384 square “wells”; these are stacked everywhere. Chemical libraries are frozen in shrink-wrapped “source plates” containing a different chemical in each well. Some are filled with natural products foraged by investigators like Clardy; other plates store compounds made by synthetic chemists like Schreiber; and others hold commercial libraries bought from drug companies or government laboratories. The small-molecule collection is always growing, and today ICCB users can choose from an estimated 250,000 different compounds.

One of ICCB’s first major discoveries involved a library of 16,320 small molecules synthesized in Russia and sold by cash-

This type of academic research, King says, “can shorten the long development cycle that you see in the pharmaceutical industry.”

strapped laboratories in the post-Soviet era. Mitchison and his collaborators were seeking small molecules to arrest cell division at a specific point in mitosis, and they cast a wide net that included the Russian collection.

Eighty-six chemicals suspended cell division at the right time, and one of them induced a structural abnormality that could be explained only by inhibition of a protein called kinesin Eg5. This compound, dubbed monastrol, became famous among biologists as the first kinesin Eg5 inhibitor and drew considerable interest from pharmaceutical companies, who believed it had great potential as an anti-cancer agent. (Mitchison reports that a number of companies are now testing compounds that have the same mitosis-arresting effect on cells as monastrol, although none of them, to his knowledge, chemically resemble monastrol.)

The ICCB has always been a technology-heavy operation that requires tending by people with eclectic skills: medical doctors and Ph.D.s, as well as self-described nerds conversant with biology, chemistry, computer science, robotics, and automation. Although major equipment manufacturers now routinely sell machines that can load experimental reagents into a 384-well plate in 12 seconds or spit out results in a few minutes, it wasn’t like that in the beginning.

The ICCB got off the ground just as Randall King finished his medical training. Two years earlier he had completed a Ph.D. in biochemistry at the University of California, San Francisco, and he felt he could do more good as a cancer researcher than as a clinician. He was immediately captivated by the mission of the new institute because it enabled “a researcher with an idea about a genetic target for a breast-cancer treatment to walk down the hall to ICCB and start the project—not just think about it.”

King became the ICCB’s first Institute Fellow, and he and a staff engineer often built gadgets that weren’t commercially available. They adapted a clunky industrial robot (made by the Seiko watch company) to manipulate 384-well plates and set up experiments. This machine is still in use, though other tinkerers

have had their way with it over the years. The arm known familiarly as “Twister” picks up assay plates loaded with cells or other biomaterials and positions them so that a second arm, equipped with an array of tiny pins that resembles a curry comb, dips first into a source plate containing 384 different small molecules, then zips across a tabletop to meticulously transfer a different chemical to each well on an assay plate.

Besides helping other scientists make good use of ICCB facilities, King uses small molecules to investigate the ubiquitin pathway, a series of enzymes that add the small protein ubiquitin to other cellular proteins to alter their function or target them for degradation by the proteasome, a large and essential structure found in every cell. The ubiquitin pathway keeps the cell functioning smoothly by tagging the right proteins at the right time for destruction—not only damaged ones, but also normal proteins that have served their purpose and need to be cleared out so the cell can continue its normal cycle. (Velcade, the first ap-

proved drug that acts on this pathway, kills multiple myeloma cells by interfering with normal proteasome activity so that proteins accumulate to toxic levels in the cancer cells.)

Seeking to learn more about the ubiquitin pathway’s role in cancer, King’s lab recently screened about 100,000 small molecules to see if they blocked cell-cycle progression. (This orderly series of events ensures that a cell, immediately before dividing in two, makes one accurate copy of its own DNA.) They embarked on this fishing expedition with no preconceived notions about which proteins might be important, an approach that leaves room for “systematic serendipity,” says King, now an assistant professor of cell biology.

Unless the proteasome itself malfunctions, the conventional wisdom was that ubiquitin-tagged proteins were certain to be destroyed. But King was surprised to see that, in some of his experiments, tagged proteins were not always consumed by the voracious proteasome. The reason turned out to be small molecules that were the chemical equivalent of Harry Potter’s cloak of invisibility: they made ubiquitin-tagged proteins impossible for the proteasome to see. Having escaped destruction, these proteins built up like uncollected garbage, choking and eventually killing cancer cells.

King calls these small molecules “ubistatins,” and readily admits they are not good drug candidates due to an electrical charge that prevents them from penetrating cell membranes. But the target they hit, a slot between two proteins in a complex system, could be used by drug companies to screen other chemicals that might be useful as medicines. This type of academic research, King explains, “can shorten the long development cycle that you see in the pharmaceutical industry.”

A different expeditionary path

WHEN KING SCREENED thousands of small molecules, looking for an interesting impact but with no idea which proteins he should target, he was marching into biological terra incognita

“We have three or four chemical biologists patrolling a hundred miles of waterfront.”

and hoping for an exciting find. Gavin MacBeath [pronounced “Macbeth”] employs a different exploration strategy, meticulously mapping the terrain and inventorying targets before committing to test any particular compounds. His imaginative approach to this task caused him, like his colleague David Liu, to be singled out by *Technology Review* as one of the world’s “top young innovators.”

MacBeath focused on a specific link in one of the cell’s many different tyrosine kinase pathways. These pathways are turned on when an environmental signal, such as a growth factor, binds to an enzyme, called a tyrosine kinase receptor, embedded in the cell surface. This enzyme activates the amino acid tyrosine, which attracts and activates one protein after another, creating a chain of events that drives the cell to divide. “Most cancers arise from problems in this circuitry,” says MacBeath, who spent two years as the first research fellow at FAS’s Bauer Center for Genomics Research and is now an assistant professor of chemistry and chemical biology. “Something goes wrong in the cell’s interpretation of environmental signals, and some defect in that circuitry tells the cell to grow and divide all the time, instead of dividing when it’s appropriate and stopping at the right time.”

When the tyrosine kinase pathway triggered by epidermal growth factor (EGF) gets stuck in the “on” position, for example, certain kinds of breast or lung cancers can result. Medicines such as Herceptin (for breast cancer) and Iressa (used to treat some lung cancers) interfere with this pathway so that tumor cells die instead of continuing to divide. The more scientists learn about how information travels this pathway in healthy and malignant cells, the better their chances for inventing potent new cancer treatments. MacBeath, therefore, is now focusing on proteins that use a structure called the SH2 binding domain to link with other proteins in the EGF tyrosine kinase pathway.

Although textbooks and scientific-review articles typically state that only four or five proteins have an SH2 binding domain, MacBeath realized that no one in fact had ever searched the human genome to see if this is true. When he did a genome-wide scan, he turned up a surprising 109 SH2 domains, each one potentially able to turn on about 200 other important players in the EGF signaling path. Sorting through more than 20,000 possible combinations to determine those that really matter—and thus might be likely targets for anticancer therapies that could turn them off—is no easy task.

The job was greatly simplified by the “protein function microarray” that MacBeath invented in collaboration with Stuart

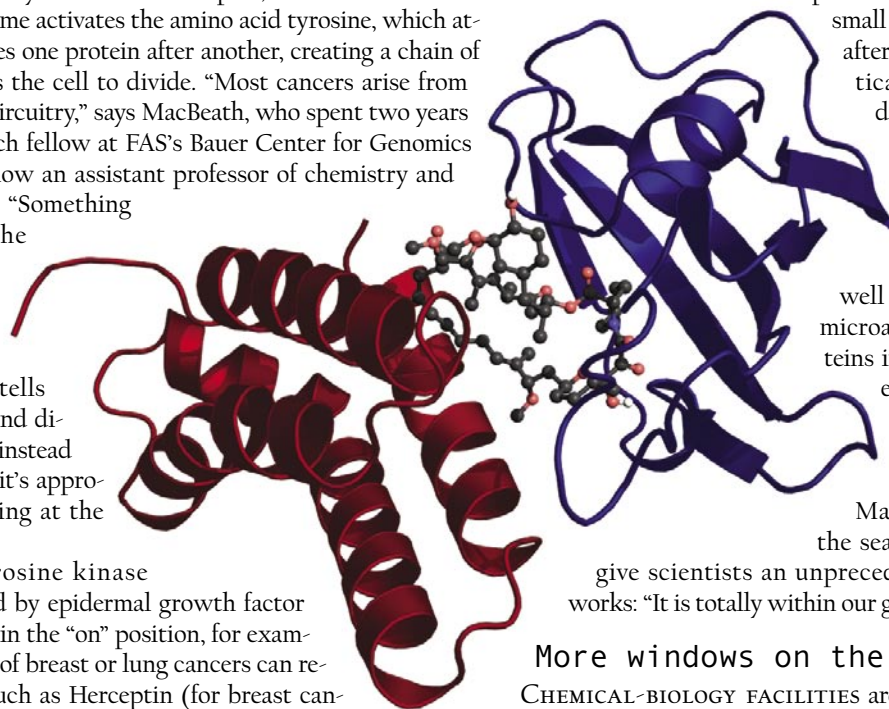
Schreiber. This technology enabled MacBeath to manufacture slides bearing purified samples of all 109 human SH2 domains, which can then be used to identify proteins in the tyrosine kinase pathway that are actually turned on when they bind with an SH2 domain. Once these are known, the next step is perturbing those interactions with small molecules to find out “how information flows through the cell under normal conditions and what goes wrong when the circuit is broken.”

This is where another invention that MacBeath had a hand in, “small-molecule printing,” proved its mettle. Small-molecule printing fixes samples of 10,000 small molecules on one glass slide after another. Hundreds of identical slides can be exposed to different proteins, generating a mind-boggling volume of data in a few hours. In addition, MacBeath and his colleagues have turbocharged the standard 384-well plate by fabricating protein microarrays that squeeze 100 proteins into each well, so that 38,400 experiments take place in a single run. Being able to screen as many as 10,000 small molecules at once, MacBeath explains, will speed the search for new medicines and give scientists an unprecedented view of how cancer works: “It is totally within our grasp to see the big picture.”

More windows on the world

CHEMICAL-BIOLOGY FACILITIES are “proliferating and expanding” at Harvard, says Jon Clardy, buoyed by support from public and private sources. In 2002, a five-year, \$40-million contract from the National Cancer Institute launched ICCB researchers on a systematic quest for molecular targets for anticancer drugs and underwrote a program that makes it possible for the facility to host non-Harvard researchers. A separate National Institutes of Health award designated HMS as one of eight Regional Centers of Excellence for Biodefense and Emerging Infectious Diseases, giving rise to a high-throughput screening operation that is now available to all qualified U.S. investigators working on drugs and vaccines against biowarfare agents. Meanwhile, a new chemical-biology facility is under construction at the Broad Institute in Cambridge, a major research enterprise launched by Harvard, MIT, and Los Angeles philanthropists Eli and Edythe Broad (see “Genomic Joint Venture,” September-October 2003, page 75). Also in Cambridge is the Laboratory for Drug Discovery in Neurodegeneration, which specializes in small-molecule discovery related to diseases such as Alzheimer’s and Parkinson’s.

In just a few years the ICCB, with its scuffed linoleum and fluorescent overheads, has gone from being the only game in town to



part of what Clardy calls the University's "high-throughput screening infrastructure." But what really signifies in the world of chemical biology, of course, is not the number of screening facilities but the caliber of the faculty members and students engaged in research. "We have incredibly creative and talented individuals, world leaders in chemical biology, all under one tent," says HMS's Christopher Walsh. "And the best thing about such people is that we are unpredictable in where we will go for new science. Our field is about multiple opportunities and multiple venues."

One place to start is the Pfizer Lecture Hall in the basement of Mallinckrodt, where 35 undergraduates gathered last fall for twice-weekly meetings of "Molecules of Life." Taking what they described as "an unconventional view of the science of living systems," Clardy and Schreiber gave lectures with titles such as "Why DNA, RNA, and proteins aren't enough" and "Designer steroids and molecular promiscuity." With pedagogical flair they introduced students to the crucial roles that small molecules play in such areas as evolution, sex, consciousness, aging, and medicine.

Several years from now, some of these students may be applying to the new Ph.D. program in chemical biology. The first group of doctoral candidates will enroll this fall, and they will be taught and mentored by faculty members drawn from the entire Harvard community. The new program is recruiting students hungry for its smorgasbord of scientific possibilities, "young chemists who want to learn biology and biologists who want to learn chemistry," says Gregory Verdine, who codirects the new program with Clardy. The Ph.D. curriculum will train leaders who won't hesitate "to cross traditional boundaries," he says, who will become major forces in academia and change discovery paradigms in industry.

"The projects that provide students with the best training are basic science, not drug development," says David Liu, a member of the University-wide committee that designed the new graduate program. Pioneering new techniques, such as his own DNA-

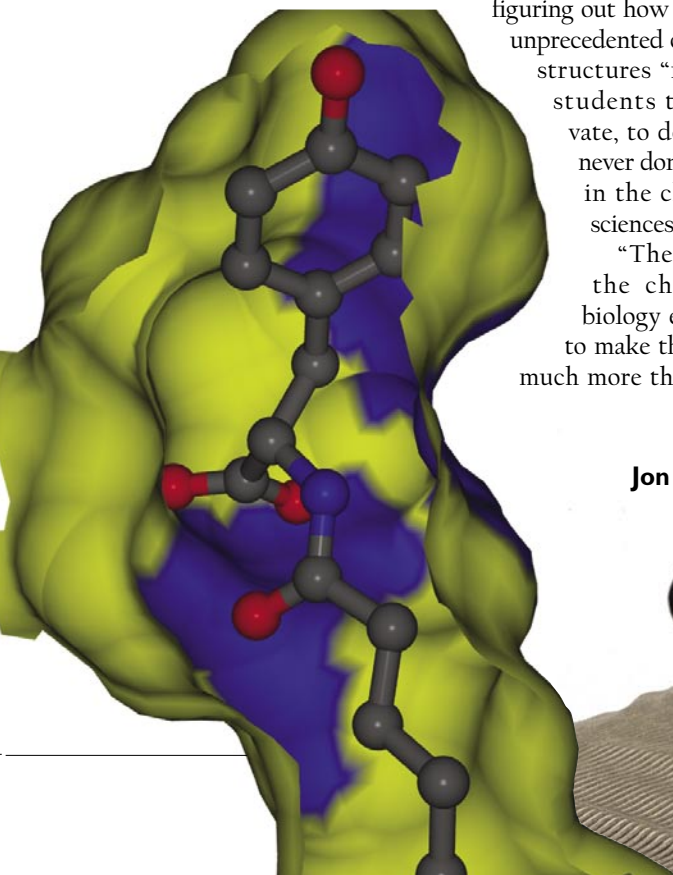
templated synthesis, and figuring out how to make unprecedented chemical structures "requires students to innovate, to do things never done before in the chemical sciences."

"The goal of the chemical-biology effort is to make the sum much more than the

parts for students, faculty, and ultimately for society," says Walsh. For veteran investigators like him, who believe that what happens in the lab today determines whether millions live or die in the coming decades, the need for more chemical biologists is critical. "Right now we have three or four chemical biologists patrolling a hundred miles of waterfront," he says. "There may be incredible opportunities two miles down the road, and there aren't enough of us to see them." ▽

Contributing editor Patricia Thomas is a Boston-based journalist and author. Her profile of computational geneticist George Church appeared in the January-February 2004 issue.

FORAGER: Jon Clardy with a rendering of an enzyme produced by DNA captured directly from soil. Microbes that live in soil are a principal source of biologically active small-molecule drugs, such as antibiotics and anti-cancer agents. **Opposite:** a rendering of rapamycin, a natural molecule produced by a soil microbe, showing how it binds two different proteins and brings them together. Clardy and Stuart Schreiber discovered how rapamycin, which is used to treat cancer, for immunosuppression in organ transplantation, and to coat the stents used to prop open blood vessels, manipulates cellular signaling. The structure shown came from one of their joint studies.



Jon Clardy

