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ENZYMOLOGIST EXTRAORDINAIRE

To create new drugs, Einstein's Vern Schramm freezes the beating heart of enzyme reactions

ENZYMOLOGIST EXTRAORDINAIRE

Vern Schramm's journey to Einstein and his contributions to science and health

BY LARRY KATZENSTEIN

Four billion years ago, when the first gene awoke within the first primordial cell and life began, that first gene may well have coded for an enzyme.

Enzymes are proteins that govern metabolism. Human cells contain some 10,000 distinct kinds. Enzymes act as catalysts, speeding reactions within biochemical pathways that perform the key tasks of life—converting food to energy, making neurotransmitters, getting rid of waste material, contracting muscles. Without enzymes, these vital reactions would occur far too slowly to sustain life.

Unfortunately, enzymes also trigger cancer, bacterial infections, hypertension and many other health problems. In fact, enzyme inhibitors account for some 30 percent of all marketed drugs, including aspirin, Viagra, cholesterol-lowering drugs and ACE inhibitors for treating hypertension. Today, as pharmaceutical research increasingly focuses on curing disease by curbing enzyme levels, a key contributor to this effort is Einstein's Vern Schramm, Ph.D., a professor of biochemistry and the Ruth Merns Chair in Biochemistry.

Dr. Schramm has pioneered the development of compounds called transition-state analogues, which inactivate enzymes involved in numerous human diseases. They bind specifically to their enzyme targets and do so thousands of times more powerfully than most other enzyme inhibitors yet developed. This binding of analogue to enzyme short-circuits disease by preventing enzymes from catalyzing their normal reactions. Such compounds have the potential to transform healthcare.

In 2017, Dr. Schramm's analogue for treating peripheral T-cell lymphoma was approved for use in Japan—the first Einstein-developed drug ever to reach the market. A transition-state analogue against the Ebola and other viruses is being developed and may soon be stockpiled for future outbreaks. More are in various stages of development, including analogues for treating stomach ulcers and antibiotic-resistant bacteria.

This article describes Dr. Schramm's analogues and what they do. It starts with the man himself: his journey from South Dakota to Einstein and to his

Clockwise from upper left: The traveling carnival operated by Vern's family; Vern at age 20; a Gilbert chemistry set; Vern at age 10; Vern, second from left, with carnival friends.

status as one of the world's pre-eminent enzymologists. He was elected in 2007 to the National Academy of Sciences, the nation's most prestigious honorary society for scientists.

SEEKING TO SOLVE THE UNSEEABLE

Ask Vern Schramm about his earliest memory and he'll take you back to a hot Midwestern summer in the early 1940s: a 3-year-old traveling with a carnival on dusty roads through South Dakota and into Iowa and Minnesota—four days entertaining folks in one small town, then moving on to another.

"The carnival was the family business—the brainchild of my maternal grandfather, who started it in 1926," he recalls. "It was a pretty big operation and needed five semitrailer trucks to transport everything. It had a Ferris wheel, several rides like the Tilt-a-Whirl with its spinning cars, many sideshows—throwing balls at a pyramid of bottles to win a teddy bear, that kind of thing—along with a few monkeys and two elephants named Judy and Alice."

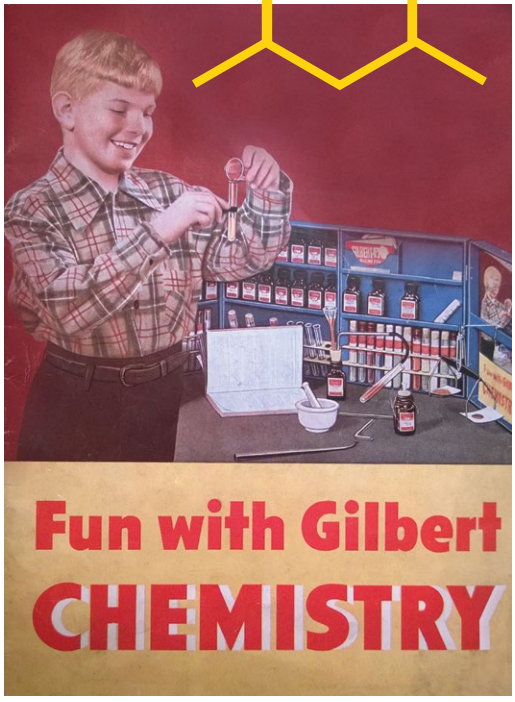


Image courtesy of the Eli Whitney Museum

The third of five children, Vern (as he likes to be called) was born in 1941 in the small town of Howard, SD (population 1,000), where the carnival was based. He was born at home, not in a hospital—standard for that time and place.

When the carnival was sold in 1946—mostly because of the strain caused by constant travel and the vagabond lifestyle—Vern's family concentrated on its electrical business. The storefront was full of televisions, washing machines and dryers, either for sale or in various stages of repair.

But for Vern, the machine shop in back was the place to be: a huge space once used for repairing carnival equipment during the winter, still filled with broken merry-go-round horses and other carnival remnants and the machines and welding torches for fixing them.

"It was an amazing place where you could do anything with metal," he says. "My brother and I had free rein to go back there and we'd weld and cut and torch. That was our playground during childhood, which I think contributed to my inventive nature."

Vern credits his interest in chemistry to his Gilbert chemistry set, a Christmas gift from his parents. "Chemistry sets in those days had compounds like sulfur and potassium nitrate and, of course, we could make charcoal ourselves. With all the ingredients for gunpowder, I helped my brother and his friend the preacher's son make Fourth of July fireworks. This inspired me to try making nitroglycerin—but fortunately I wasn't a very good chemist at age 12 and never blew anything up. But I certainly tried."

GO EAST, YOUNG MAN

His other passion was reading. "I read all the time when I was in high school," Vern says. "I read encyclopedias, books about chemistry, the classics—almost all the books in our high school library." He was not a good student, however. "I liked to learn but didn't like to study," he recalls.

Vern attended the local college, the South Dakota State College of Agricultural and Mechanic Arts, where, not surprisingly, he majored in chemistry. But in his junior year, he was offered a research assistantship and free tuition if he'd switch to a microbiology major. He stayed on after graduating, working full-time as a technician with no plans for the future. Then came the phone call that changed his life.

"One day at school I got a call from someone at the Harvard School of Public Health wondering if I'd like a fellowship to complete a master's degree in the department of nutrition. I said 'Yeah, okay.'" Unbeknownst to Vern, South Dakota State's chair of microbiology had recommended him to a good friend who worked in the department.

"Going from South Dakota State to Harvard was like moving to outer

space," says Vern, who—aside from his forays with the family carnival—had ventured out of state just once before.

As Vern was finishing his master's degree, he spotted an ad on a library bulletin board. The Australian National University (ANU) was seeking Ph.D. students. By then he had married Deanna, a South Dakota State classmate, and the couple had an infant daughter. His application to the Ph.D. program in biochemistry was accepted, and he and his family headed for Canberra, Australia.

John Morrison, the ANU professor who'd informed Vern of his acceptance and took him on as a grad student, was a highly respected enzymologist. His special interest was tight-binding enzyme inhibitors, which would become a focus of Vern's own scientific career.

After earning his Ph.D. in biochemistry, Vern returned with his family to the United States, where he did a postdoctoral fellowship at NASA's Ames Research Center in California. He then got a faculty position in biochemistry at Temple University Medical School in Philadelphia. There he studied the mechanisms by which enzymes transform the molecules they act on, known as substrates, into entirely different molecules called products, and how those reactions occur so incredibly rapidly.

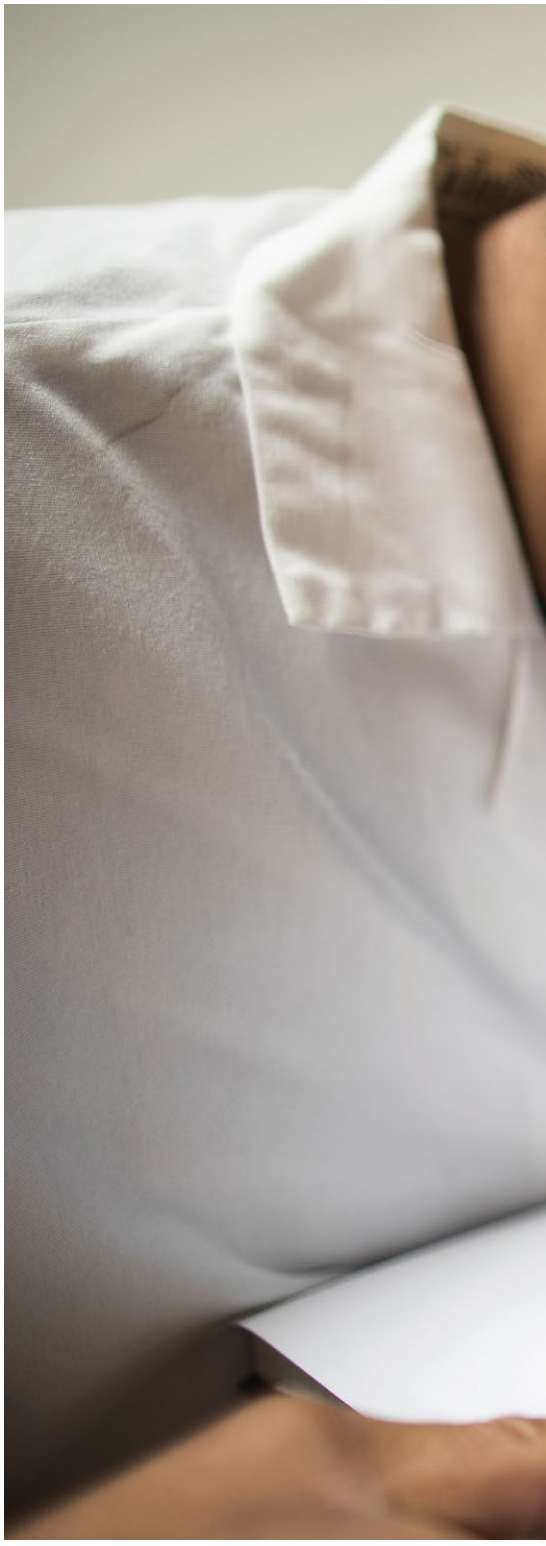
NATURE'S VITAL CATALYSTS

Enzymes have evolved over millions of years to bind to certain substrates and catalyze reactions with optimal efficiency—typically 10^{10} to 10^{15} times faster than would be possible without them. An enzyme's three-dimensional shape—its conformation—results from its amino-acid sequence. That shape, in turn, determines the enzyme's specificity: the particular substrates it can physically attract.

Enzymes have evolved over millions of years to bind to certain substrates and catalyze chemical reactions with optimal efficiency.

An enzyme molecule will bind to and interact with one substrate molecule after another. But only a small portion of the enzyme—its active site—binds the substrate molecule tightly enough to catalyze an enzyme reaction. The enzyme's active site bristles with catalytic groups that break the substrate's chemical bonds and rearrange its atoms to form a chemically different product. But for catalysis to occur—for substrates to become products—a structure called the transition state must form during the enzyme reaction.

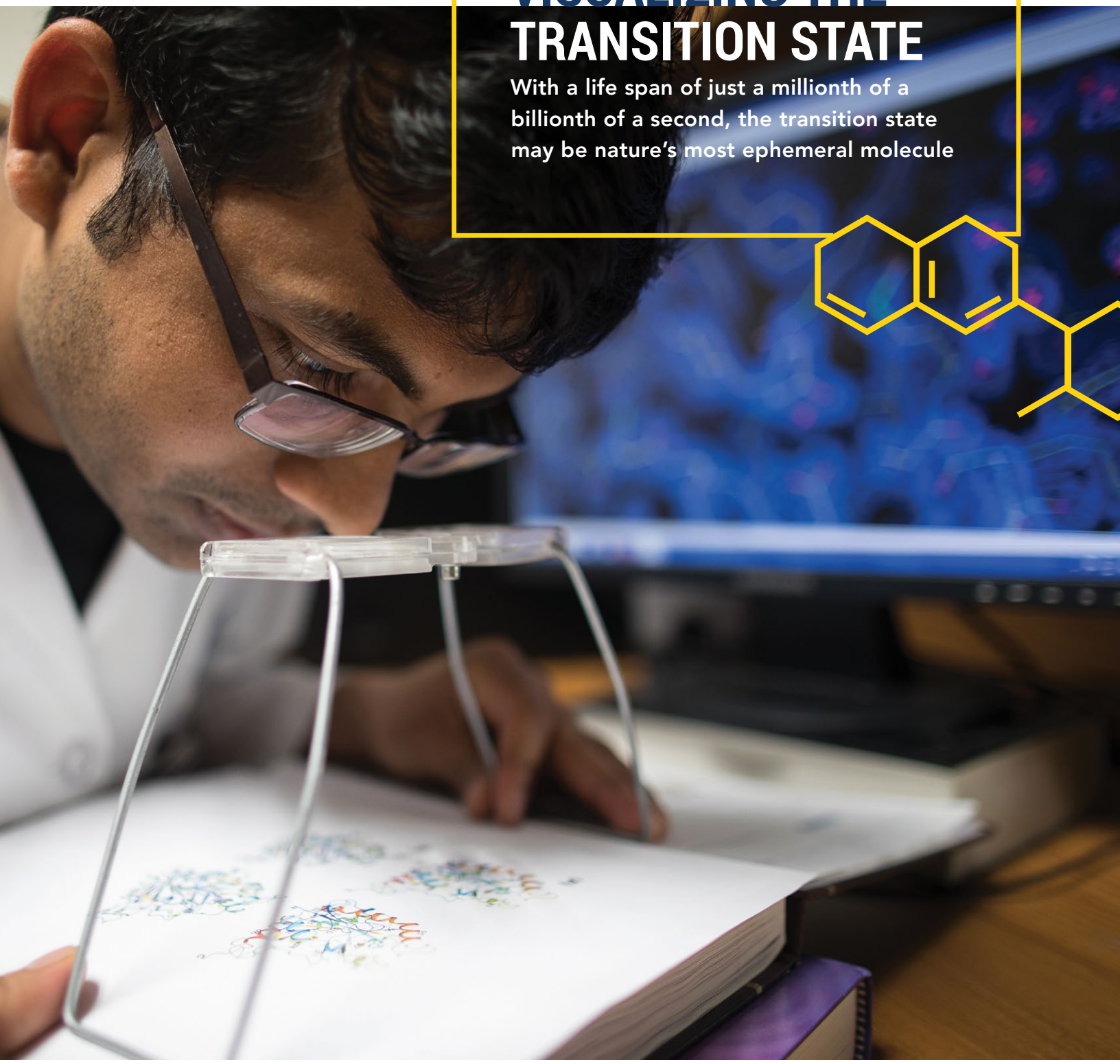
Seventy years ago, two-time Nobel laureate Linus Pauling hypothesized that enzymes bind tightly to substrates during transition states, which accelerate the conversion of substrates into products. Vern's great achievement was finding a way to visualize transition states' incredibly brief lives—they exist for just a millionth of a billionth of a second—and use that information to attack some of humankind's most intractable diseases.



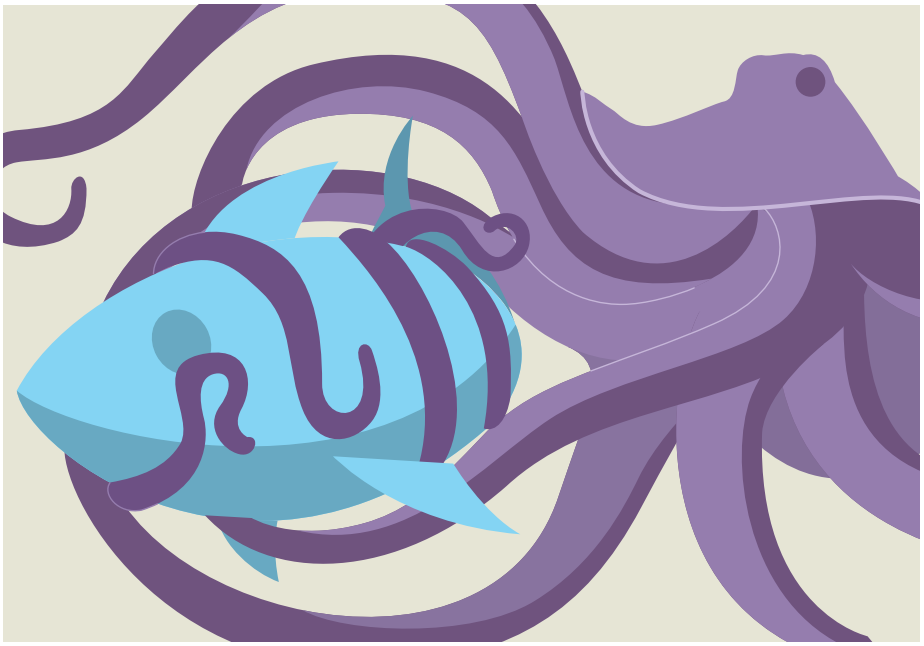
At right: Rajesh Harijan, Ph.D., a postdoctoral fellow in Vern's lab, examines enzyme-inhibitor molecules.

VISUALIZING THE TRANSITION STATE

With a life span of just a millionth of a billionth of a second, the transition state may be nature's most ephemeral molecule



ENZYMOLOGIST EXTRAORDINAIRE



© Margaret Nielsen

Grasping the transition state: Picture an octopus that explores a captured fish with all eight tentacles. The tighter the collective grip, the faster the fish can be swallowed.

GRASPING THE TRANSITION STATE

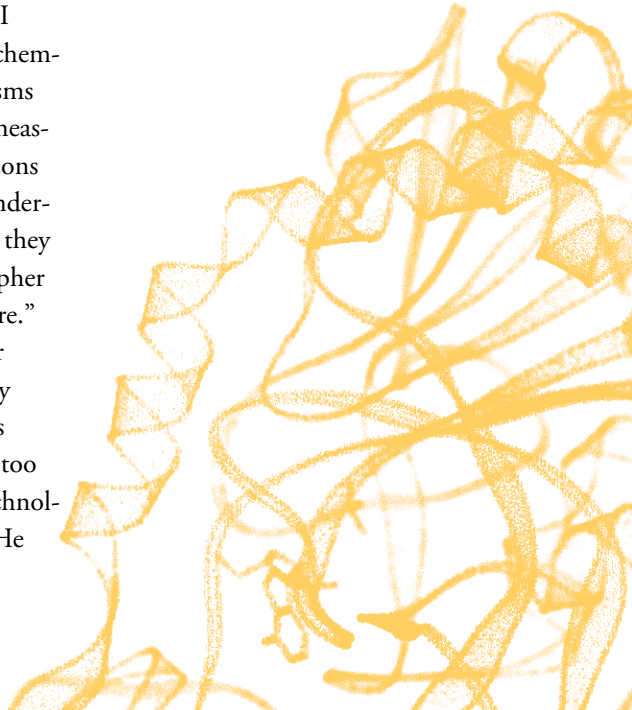
Picture an octopus that has captured a fish and explores it with all eight tentacles, seeking the optimal combination of grips to immobilize it: the tighter the collective grip, the faster the fish can be swallowed. Something similar happens when an enzyme captures a substrate molecule and clasps it to its active site.

Instead of tentacles, the enzyme's active site uses its catalytic groups to explore the surface of the captured substrate, seeking interactions tight enough to break its chemical bonds and catalyze the reaction. Finally—and only when multiple interactions have reached optimal strength at exactly the same time—comes the transition state: a swirl of atoms and electrons amidst chemical bonds that are breaking and releasing energy to speed the reaction to completion. The fleeting transition state that forms is neither substrate nor product, but rather a ghostly combination of both of them.

The transition state is the beating heart of enzymology—and of life: Its formation provides the essential chemical changes needed for all of biology's metabolic functions. But what did these short-lived structures look like? Vern was well suited to solve that mystery.

"Ever since I was an undergraduate, I'd been interested in how enzymes worked and how they perform amazing chemical reactions so hard to achieve using organic chemistry," he says. "I wanted to know about the atomic chemistry that governs enzyme mechanisms and investigate enzyme kinetics—measuring how changing certain conditions alters reaction rates. I felt that to understand enzymatic reactions and how they proceed so fast, you needed to decipher the transition state's atomic structure."

Vern began that effort soon after arriving at Temple in 1971. Directly observing transition-state structures was impossible: They disappear far too quickly to be seen with imaging technology such as X-ray crystallography. He



How to Freeze
A FEMTOSECOND

Transition states last for a millionth of a billionth of a second—far too brief a period to be observed directly. Vern knew that two features are needed to describe all molecular interactions in biology: their geometric shape and the distribution of their electrons. He realized he could use isotope effects to describe both features and, by doing so, solve the transition-state structures of enzymes.

Isotopes are different-mass versions of naturally occurring atoms, such as the hydrogen, carbon and nitrogen atoms that typically form substrate molecules. An ordinary hydrogen atom, for example, has an atomic weight of one (one proton), while its isotope deuterium has an atomic weight of two (one proton plus one neutron). Isotopes soared to the forefront of scientific consciousness after World War II: tritium, for example—hydrogen's heaviest isotope, with an atomic weight of three—was a key ingredient in the hydrogen bomb.

Kinetic isotope experiments involve taking the substrate of an enzyme reaction and replacing its hydrogen, carbon and nitrogen atoms with heavier isotopes. Running those mass-altered substrate variants one at a time through the enzymatic reaction gives an atom-by-atom readout of how those atoms respond to the transition state.

"Each atomic substitution alters the substrate's bond-vibrational frequency in the transition state, causing a small but measurable change in the reaction time compared with the time required when the enzyme reacts with normal, 'unsubstituted' substrate molecules," Vern says. "Those isotope effects on the reaction rate are exquisitely difficult to measure, but I love doing that analytical chemistry work."

Combining the results of those isotope-effect experiments provided crucial insights into the transition state's geometric shape and electron distribution—information needed to deduce the transition state's atomic structure. The final step: Use computational quantum chemistry to search through thousands of the enzyme's theoretically possible transition states to find the model that most closely matches the experimental results from kinetic isotope studies.

"That structure is the most complete picture of an enzyme's transition state that we can get," Vern says. "Now that we have a blueprint of the transition state, we can design stable transition-state analogues that will mimic its structure and, we hope, powerfully inhibit that enzyme."

READ MORE
Learn how persistence paid off for Vern, who used isotopes to find transition states despite the skepticism of his peers:
magazine.einstein.yu.edu/isotopes18

realized that the only way to "see" transition states was by measuring kinetic isotope effects: Swapping out a substrate's hydrogen, carbon and nitrogen atoms for their slightly heavier isotopes and observing how those slight changes alter the substrate's reaction with its enzyme.

In 1984, Vern and his colleagues published their first-ever transition-state structure, for the enzyme AMP nucleosidase. Over the next few years they solved transition states for half a dozen more. "This was purely fundamental research—science at its most basic," he says. "Back then we'd given no thought to designing drugs." That would come later, when Vern saw the therapeutic potential in targeting a single enzyme.

REVELATIONS FROM A RARE DISEASE

Each year several children in the world are born healthy but by age 3 or 4 become helpless against bacterial or viral infections because their infection-fighting T cells have disappeared. In 1975, University of Washington hematologist Eloise Giblett discovered the cause: A genetic defect meant the children couldn't synthesize the enzyme purine nucleoside phosphorylase (PNP). Without PNP, the children's T cells couldn't multiply to fight infections, which overwhelm the limited number of T cells available to combat them.

Dr. Giblett's discovery meant that children born with this condition could be saved through stem-cell transplants that normalize PNP levels. It also showed that *purposely* knocking out PNP could be a useful strategy against diseases caused by excess T cells, especially since these children weren't otherwise harmed by their lack of PNP. Vern and other scientists realized that a drug specifically targeting PNP would have tremendous health implications.

Two types of blood cancer—T-cell leukemia and T-cell lymphoma—result from rapidly dividing T cells. In addition, most of the more than 70 autoimmune diseases are caused by dividing T cells that mistakenly attack a person's own tissues. A drug that specifically inhibits PNP would presumably work against all of them.

Also in the 1970s, enzymologist Richard Wolfenden at the University of North Carolina was working on a strategy for inhibiting enzymes. He'd followed up on Linus Pauling's idea that enzymes bind tightly to their substrates during the transition state and was writing equations to show how that happens. When substrate and enzyme are together at the transition state, his equations predicted, the energy buildup that occurs as the substrate's bonds are broken will dramatically increase the strength of enzyme-substrate binding by a factor of 10^{10} to 10^{15}. The tighter the binding at the transition state, the faster the reaction can proceed.

If scientists could develop what Wolfenden dubbed transition-state analogues—compounds combining features of the substrate, the transition state and the product—something else should occur: In a sea of thousands of substrate molecules, an enzyme molecule would preferentially seek out a lone transition-state analogue molecule, enticed by the prospect of forming a transition-state structure with what resembles a substrate molecule.

But unlike a "real" substrate molecule, the analogue resists chemical change when the enzyme binds to it. So now the enzyme—rather than propelling a chemical reaction—finds itself part of a stable duo: imprisoned by the analogue and bound to it millions of times tighter than if the enzyme had met up with an actual substrate molecule.

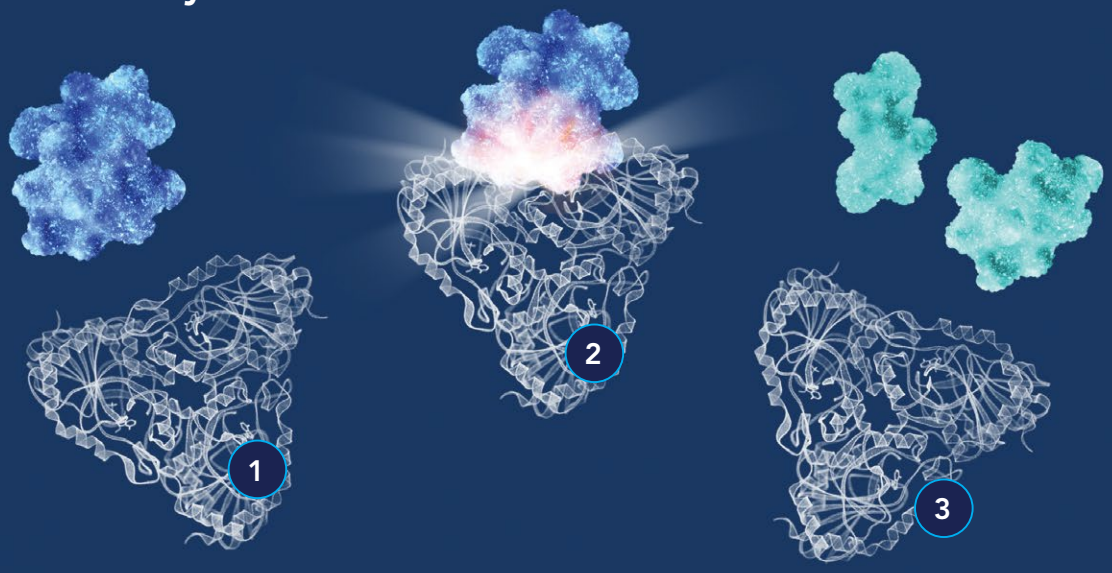
"Wolfenden's work set the stage for the search for transition-state analogues," Vern says. "But until we started our research there was no way to understand what an enzyme's transition state looked like and therefore no way to rationally design stable transition-state analogue molecules that mimic it. We realized that solving the transition state for any enzyme would put us in a good position for designing analogues to inhibit it, including PNP."

In 1987, Einstein recruited Vern to chair its biochemistry department. He agreed to take the job while continuing his research. He and his colleagues solved PNP's transition-state structure in 1993. The next step—using that structure as a blueprint for synthesizing transition-state analogues for use as drugs—would prove to be formidable. Luckily, chemists halfway around the world were looking for a challenge.

The chemists worked at Industrial Research Ltd., a government research institute in Lower Hutt, New Zealand. One day in 1991 they were visited by Paul Atkinson—a former Einstein biology professor and native New Zealander. He'd recently moved back home to direct AgResearch, a New Zealand government research facility. He soon learned that another research team—the Carbohydrate Chemistry Group—was looking for new partners.

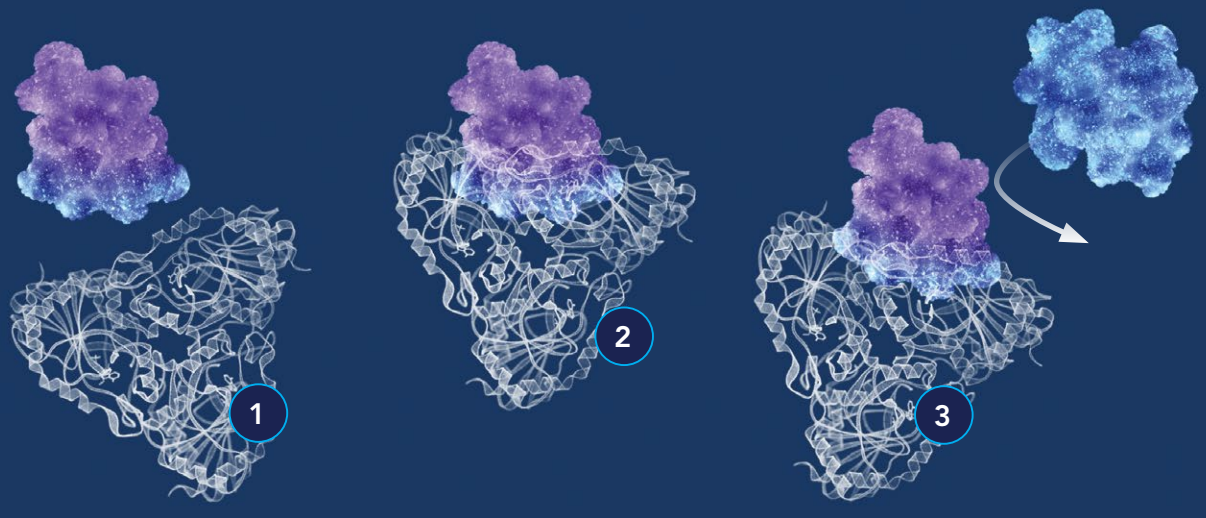
"Paul knew about my work here at Einstein," Vern says, "so he told the team leader, 'You should go talk to Vern Schramm. He's doing some interesting carbohydrate chemistry that you might want to get involved with.'"

A Normal Enzyme Reaction



(1) An enzyme molecule's active site is attracted to and binds with a substrate molecule (blue); a transition state then forms (2) to accelerate the substrate's conversion into a product or products (3).

What a Transition-State Analogue Does



Transition-state analogues inhibit the enzyme reactions that cancer cells, parasites and other disease-causing cells depend on. (1) The enzyme molecule is attracted to what appears to be a substrate molecule but is actually a transition-state analogue (blue-purple molecule); (2) the analogue binds the enzyme millions of times more tightly than a normal substrate molecule would, because the analogue's bond-breaking energy is converted to binding energy; (3) with the enzyme's active site permanently blocked by the analogue, it can no longer react with substrate molecules.

THE FIRST MARKETED EINSTEIN DRUG

Mundesine's development resulted from an unusual and ongoing research collaboration among scientists on opposite sides of the world—in the Bronx and in New Zealand

Two of the New Zealand chemists arrived in the Bronx a year later. "We drove to a waterfront restaurant in New Rochelle," Vern recalls. "Over drinks, I sketched on a bar napkin the structure of a molecule I thought would be a good transition-state analogue for PNP. The concept of transition states was new to them, but they understood the chemistry needed to synthesize the inhibitor I wanted. We shook hands and agreed to work together, sharing equally in any revenue generated."

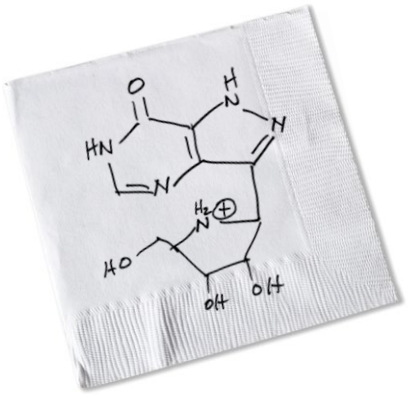
Back in New Zealand, synthesizing what became known as "the bar-napkin inhibitor" was a difficult four-year effort requiring 21 steps. Finally, the first PNP transition-state analogue was successfully synthesized, nearly identical in structure to Vern's bar-napkin sketch.

In 2000, Einstein licensed the analogue to a commercial partner, BioCryst Pharmaceuticals, Inc., for testing against blood cancers. BioCryst took the analogue through phase 2 human trials and then sublicensed it to Mundipharma, which brought it through pivotal clinical trials leading to regulatory approval. This first of Vern's PNP inhibitors, now known as Mundesine®, was approved in Japan in 2017 for treating advanced cases of peripheral T-cell lymphoma.

It took 20 years for Vern's PNP inhibitor to become the approved drug Mundesine. Ironically, the drug's potency prolonged its approval process. "Pharmaceutical companies didn't understand how to use transition-state analogues at first," he says. "There was a big learning curve before they realized just how effective these compounds are at very low doses."

Indeed, just a few milligrams of Mundesine stop T cells throughout the body from dividing. But it's their specificity that makes Mundesine and other transition-state analogues unique.

Ordinary chemotherapies can cause serious side effects by killing dividing cells, both normal and cancerous, throughout the body. But Mundesine affects only rapidly dividing T cells that cause blood cancers. Mundesine starves them of PNP, the enzyme T cells need to get rid of the chemical 2'-deoxyguanosine, which accumulates to lethal levels without PNP. Thanks to its highly specific mode of action, Mundesine is well tolerated and causes few serious side effects; it's one of the few anticancer agents that can make that claim.



Vern sketched on a bar napkin the molecule he thought would work to inhibit the enzyme PNP. Mundesine, approved in Japan in 2017, closely resembles Vern's original sketch.

Opposite: Vern (second from left) with his New Zealand collaborators, from left: Peter Tyler, Ph.D., Gary Evans, Ph.D., and Richard Furneaux, Ph.D.

When a Drug Becomes A GIRL'S LAST HOPE

Basic scientists like Vern rarely know if their work will affect human health. "Fundamental discoveries are usually single steps in a long path toward applications," Vern says. "Typically you wouldn't even know if you've contributed to a new drug." But Vern was lucky: long before Mundesine was approved for use in Japan, he learned that the drug he designed could save lives.

Mundesine's approval in Japan for T-cell lymphoma came after 19 clinical trials that spanned 10 years and involved about 500 patients with several types of cancer. The trials were intended for adults; but an early exception was made for a 2-year-old California girl (at right) with T-cell leukemia, for which Mundesine has not yet been approved.

Photo courtesy of Jeff and Linda Lambertson

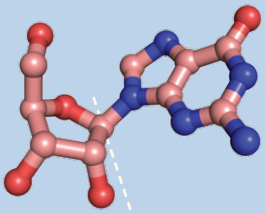
"We heard that the girl—we didn't know her name—had failed all standard therapies for T-cell leukemia," says Vern. "As a father, I could imagine how devastating that must be for a family. The company sponsoring her trial received periodic reports from her grateful dad, and their emotional impact exceeded anything else I'd experienced as a scientist." Vern finally learned more about the successful treatment of the girl, Katie Lambertson, from a 2014 magazine article.

The 10-Step Recipe for Making

ENZYME INHIBITORS

TARGET AN ENZYME ...

associated with human disease. Make sure that inhibiting this enzyme will not disturb normal physiological processes.



1

LABEL THE SUBSTRATE ...

with isotopes to reveal vibrational changes in its chemical bonds during the reaction's transition state.

2

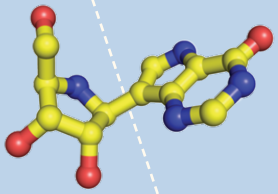
ISOLATE AND PURIFY ...

the enzyme to be studied.

3

DESIGN AN ANALOGUE STRUCTURE ...

that resembles the transition-state blueprint.



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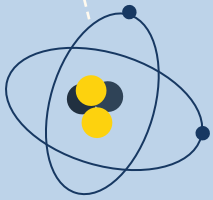


USE COMPUTATIONAL QUANTUM CHEMISTRY ...

to convert the kinetic isotope results into a blueprint of the transition-state structure.

5

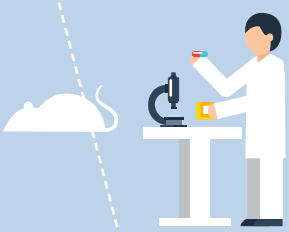
Following a tried-and-true recipe, Vern and his New Zealand colleagues have solved the transition states for some 30 enzymes and have developed analogues for about half of them.

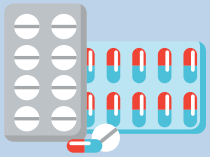
The packaging for Mundesine tablets, now on the market in Japan.

Photos courtesy of Mundipharma

DO LAB TESTING ...

to make sure the transition-state analogue can inhibit the enzyme.





LICENSE THE ANALOGUE ...

to a pharmaceutical company that will advance it through human trials.

6 7 8 9 10

COLLABORATE WITH COLLEAGUES ...

to synthesize the analogue.

LEARN WHETHER THE ANALOGUE ...

can penetrate the appropriate cells to inhibit the target enzyme and affect cells in the desired way.

POP THE CHAMPAGNE ...

when the analogue is approved for treating human disease.

FURTHER USES FOR ENZYME INHIBITORS

By targeting PNP and other enzymes, Vern and his colleagues have created powerful compounds for potential use against a variety of microbes, including the Ebola virus

Over the past 15 years, Vern and his New Zealand colleagues (now at the Ferrier Research Institute of Victoria University of Wellington) have developed even more powerful second- and third-generation transition-state analogues. They're intended for use against the numerous health problems traceable to PNP and other enzymes.

• **From STDs to Deadly Viruses.** A PNP inhibitor that failed against a parasite has emerged as the first broad-spectrum antiviral agent, effective against some of the world's most lethal viruses.

The protozoan parasite *Trichomonas vaginalis* seemed a perfect target for a PNP inhibitor. It causes trichomoniasis, a common sexually transmitted disease. And the parasite's unique version of PNP meant that inhibiting it wouldn't harm people.

"We synthesized a transition-state analogue we called Immucillin-A," Vern says. "It did a terrific job inhibiting the enzyme but failed to kill the parasite." PNP, it turned out, was not essential for the parasite's survival. But later, when BioCryst tested all its Einstein-licensed drugs against viruses, Immucillin-A prevented more than 20 RNA viruses from multiplying, including Ebola, Marburg, Zika and yellow fever.

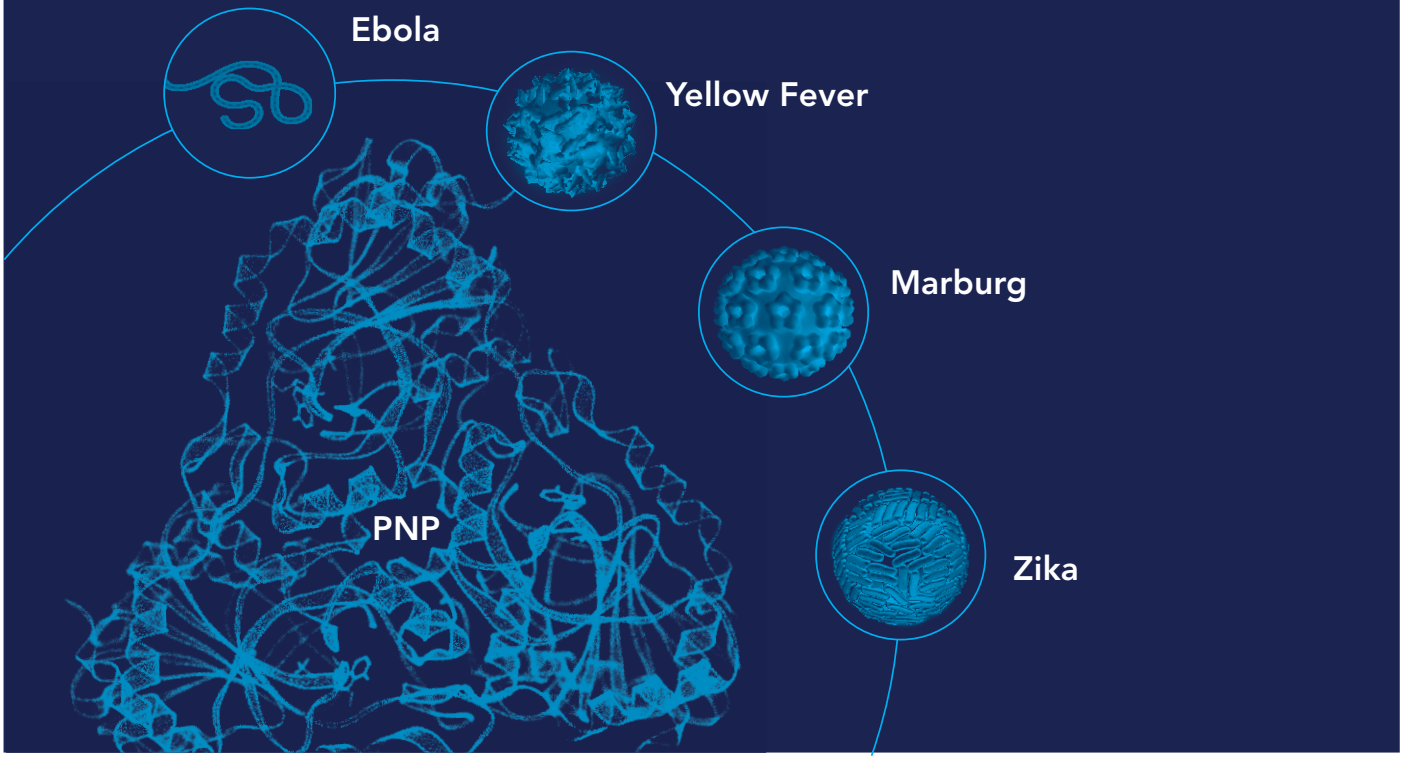
Immucillin-A doesn't actually function as a transition-state analogue against viruses. Instead, virus-infected cells absorb the analogue and metabolize it into RNA building blocks that are defective; replicating viruses unwittingly use the defective RNA building blocks, which sabotage their efforts to multiply.

"That was something we never could have predicted from our own work," Vern says. "I credit Einstein's office of biotechnology and business development, which Executive Dean Ed Burns created in 2000, for finding commercial partners like BioCryst to give our discoveries the best possible chance of being turned into drugs."

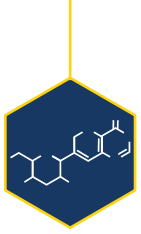
BioCryst teamed with United States government agencies to further develop the analogue, now called galidesivir. It was found safe when given intramuscularly to healthy human volunteers.

A Fortuitous Finding Goes Viral

An inhibitor designed to target the enzyme PNP in *Trichomonas* works in a totally different way to prevent deadly viruses from multiplying.



Olivia Acland / Barcroft Images / Barcroft Media via Getty Images



ENZYMOLOGIST EXTRAORDINAIRE

BioCryst has reported the results of studies in which nonhuman primates were infected with lethal doses of the Marburg or Ebola viruses. Forty-eight hours later, they received injections of galidesivir—which allowed them to survive the otherwise fatal infections. Galidesivir also demonstrated antiviral effects in nonhuman primates infected with the Zika virus.

Following more studies involving animals infected with Ebola and Marburg, galidesivir will be evaluated in additional human safety trials. If those tests are successful, galidesivir may then be manufactured and stockpiled for use in future outbreaks of the viruses.

• **Malaria.** While Ebola is scary, malaria—caused by single-celled parasites belonging to the *Plasmodium* genus—causes many more deaths: an estimated 429,000 per year.

Vern targeted *Plasmodium falciparum*, the deadliest malarial species, by exploiting its Achilles' heel: it can't directly synthesize purines, the vital building blocks for making DNA. Instead, the parasites need their own version of PNP to make hypoxanthine, which they then convert to purines. Inhibiting *P. falciparum*'s PNP would cut off its supply of hypoxanthine—and deprive the parasite of the purines it needs to survive. A PNP inhibitor, now called BCX4945, was developed to do just that.

BCX4945 proved effective against laboratory cultures and was then tested on three nonhuman primates infected with a strain of *P. falciparum* that is lethal without antimalarial therapy. When orally administered twice a day for seven days, BCX4945 cleared the infections from all the animals between the fourth and seventh day of treatment. No signs of toxicity were observed.

Results were announced in 2011, but the inhibitor has yet to be evaluated in human trials. "It's frustrating to have a potential cure for malaria sitting on the shelf," Vern says, "but the downside of licensing your compounds is that you lose control over which ones are developed. Companies are sometimes reluctant to spend hundreds of millions of dollars on human trials if sales can't replace development costs."

RESISTANCE-FREE ANTIBIOTICS

In a 2014 report, the World Health Organization called microbial resistance to antibiotics "an increasingly serious threat to global public health" and offered this warning: "A postantibiotic era—in which common infections and minor injuries can kill—is a very real possibility in the 21st century."

By wiping out most microbes they encounter, standard antibiotics actively select for antibiotic-resistant strains. The few microbes that inevitably survive the antibiotic onslaught can multiply and thrive in a milieu free of competitors. Survivors transmit their resistance traits to succeeding generations, requiring ever more potent antibiotics, leading to bacterial strains that show even greater resistance: a vicious and dangerous cycle.

In Vern's lab, a top priority is developing new antibiotics—enzyme inhibitors that "disarm" rather than destroy disease-causing bacteria.

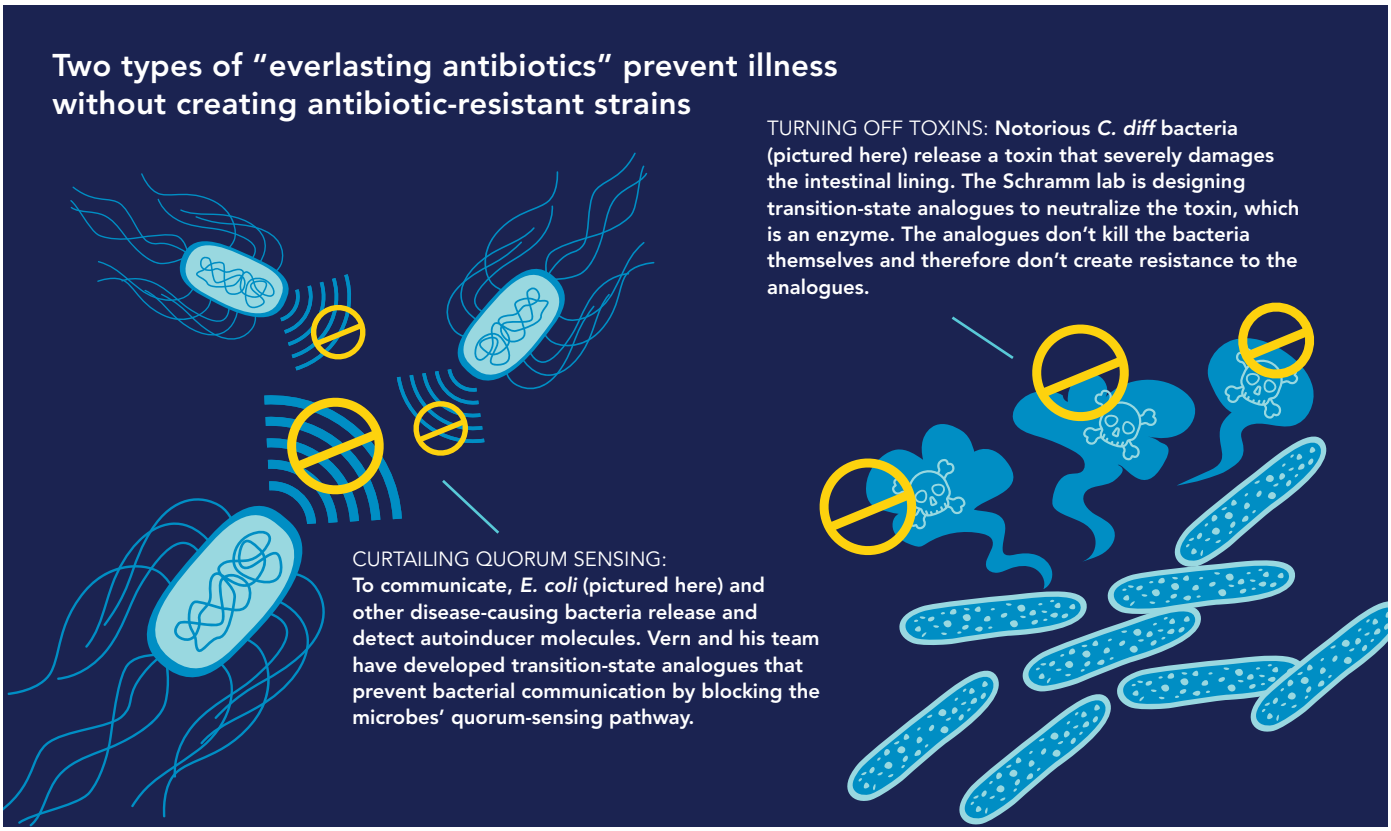
Dubbed "everlasting antibiotics," these drugs could treat infections without exerting the selective pressure that produces antibiotic-resistant strains. One type works by sabotaging communication among bacteria, and another by neutralizing disease-causing toxins. "Bacteria disarmed in these ways will simply join the body's billions of other harmless bacteria," Vern says.

• **Quelling Quorums.** Individual bacteria produce and detect signaling molecules called autoinducers that tell them how many of their colleagues are nearby. Sensing a high number of autoinducers tells disease-causing bacteria that their colleagues are present in sufficient numbers (i.e., a quorum) to change from bystander to virulent mode—attacking their hosts by releasing toxins and forming slime-coated, hard-to-treat biofilms, responsible for infections that often afflict people who have indwelling catheters.

"We hypothesized that blocking the quorum-sensing pathway in bacteria would cut the telephone wires and prevent them from communicating with each other," Vern says. He knew that the bacterial pathway for synthesizing autoinducers required an enzyme called MTAN, so he and his colleagues designed and developed a family of transition-state analogues to target it.

The MTAN inhibitors were cultured overnight with the toxin-forming bacterial species *Vibrio cholera* (the species that causes cholera) and *E. coli 0157:H7* (a potentially lethal strain of *E. coli*). The inhibitors disrupted quorum sensing in both species and significantly reduced biofilm production—without killing the bacteria. "Quorum sensing isn't essential for survival of these bacteria," he says. "So they're not killed by our MTAN inhibitors and therefore shouldn't develop resistance to them."

To see if bacteria might eventually develop resistance, Vern's lab grew 26 successive generations of the *Vibrio* and *E. coli* bacteria, all of them cultured with one of the quorum-sensing inhibitors. "The 26th generation of both bacterial species was just as sensitive to the



inhibitor as the first was," he says.

• **Turning Off Toxins.** The intestinal bacterium *Clostridium difficile* can be lethal and is difficult to treat. Infections with *C. diff*, as it's called, predominate in hospitals, where antibiotic use is common. Today's antibiotics can deplete much of a person's healthy gut microbiome but usually don't eliminate *C. diff*, which can then flourish in the gut. Ever more powerful antibiotics have recently spawned increases in highly virulent, antibiotic-resistant *C. diff* strains.

C. diff sickens about half a million Americans yearly and causes about 30,000 deaths. Illnesses and deaths occur because *C. diff* releases toxins that damage the gut wall, leading to diarrhea and potentially fatal colitis. Toxin B, the major tissue-damaging toxin, is an enzyme. Vern and his team are designing transition-state analogues against *C. diff*'s toxin B and not the bacteria themselves. "*C. diff* would not know that these antitoxin compounds are present," he says, "so there's no pressure on the bacteria to develop resistance."

SAFE TREATMENT FOR ULCERS

In 1982, Australian scientists Barry J. Marshall and J. Robin Warren reported that a previously unknown bacterial species called *Helicobacter pylori* causes most cases of stomach ulcers and duodenal (small-intestine) ulcers, which had long been thought to result from stress or other lifestyle factors. The discovery earned Marshall and Warren the 2005 Nobel Prize for Physiology or Medicine and transformed ulcers from a chronic, often disabling condition to a bacterial disease curable with antibiotics.

Over the past 30 years, however, *H. pylori* has become increasingly resistant

> "The *C. diff* bacteria would not know that these antitoxin compounds are present, so there's no pressure on the bacteria to develop resistance."

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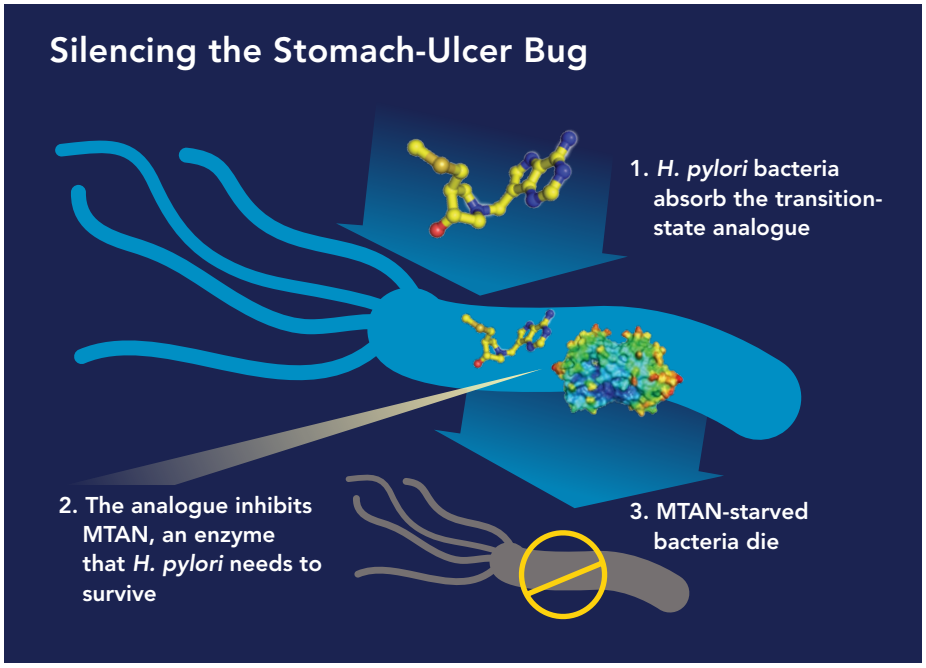
ENZYMOLOGIST EXTRAORDINAIRE



to antibiotics. Even today's extra-intensive antibiotic regimen fails to eradicate *H. pylori* in 20 percent of patients. Moreover, antibiotics play havoc with patients' normal gut microbiome, clearing the way for *C. diff* to establish itself.

A 2017 study that followed 260 stomach-ulcer patients treated with *H. pylori* eradication therapy found a "significant rate" of *C. diff* colitis: 12 of the patients—4.6 percent of the total—developed the often-fatal illness. Antibiotics that eliminate *H. pylori* without putting patients' lives at risk were clearly needed.

In the midst of his quorum-sensing research targeting the enzyme MTAN, Vern read that *H. pylori* has a very unusual metabolic pathway. It depends on MTAN not for quorum sensing but for survival. "We realized our MTAN inhibitors that halted quorum sensing in other bacteria could work as antibiotics against *H. pylori*," he says.

In research published in 2015 in the *Journal of the American Chemical Society*, he and his colleagues reported that 10 of their MTAN inhibitors were up to 2,000 times more powerful at preventing *H. pylori* growth than many antibiotics now used to treat the infections.

"We think our MTAN inhibitors will capture the market for treating stomach ulcers," he says. "They're far more potent than existing *H. pylori* antibiotics, and our studies found they don't harm normal gut bacteria and so won't increase patients' risk for developing *C. diff* infections—a major drawback to current ulcer therapy." Several of those inhibitors were recently licensed to a biotech company interested in developing them into *H. pylori* drugs.

THE ROAD AHEAD

Modern medicine emphasizes finding drugs that target enzymes. Yet when it comes to developing drugs using transition-state analysis, Vern and his New Zealand colleagues have few rivals—which is surprising, because the strategy epitomizes rational drug design.

"Companies designing enzyme inhibitors typically start by searching through millions of compounds in their chemical libraries, hoping they'll chance on one that inhibits their target enzyme," Vern says. "They'll find one that works pretty well and spend years refining it for clinical trials. We start by identifying the enzyme we want to target. Once we solve its transition state, we synthesize a transition-state analogue to mimic it. If we've done things correctly, that analogue becomes the compound that will go into clinical trials."

His different approach, he notes, "requires many cutting-edge scientific technologies that pharmaceutical companies find daunting." But he predicts they'll see the light. "If we get three or four drugs FDA-approved using this process, which could occur in the next decade, transition-state analysis will become a standard procedure that pharmaceutical companies will use to design and develop new drugs."

As for his own research, he says, "the enzymes we're now targeting are posing some very tough challenges—transition states that are hard to solve and analogues that will be difficult for our New Zealand colleagues to synthesize." But he thrives on such challenges.

"You have to be optimistic as a scientist," Vern says. "There are always four reasons to think an experiment might fail, but you've got to do the experiment anyway and be prepared for all those failures before you do the correct one. That sense of optimism helps us move forward. There are a lot more enzymes out there that we need to target." E

At left: Yacoba Minnow, a Ph.D. student in Vern's lab, carries out pipetting for an experiment.