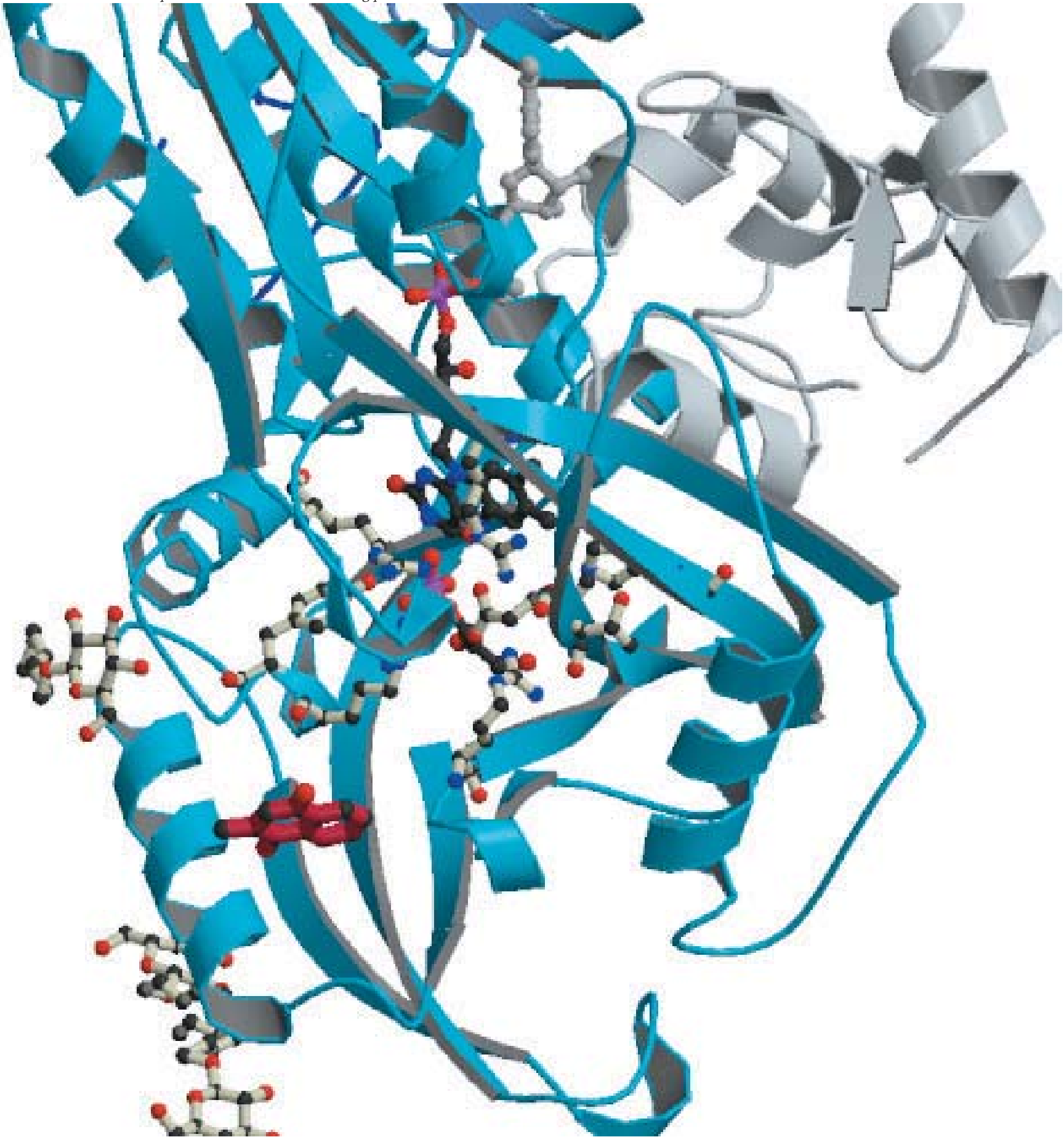


INVESTIGATIONS

Explorations and revelations taking place in the medical school



A LIST OF POSSIBILITIES

YEH LONG

A MISSING LINK IN CHRONIC ILLNESS

BY ELAINE VITONE

In recent years, researchers have begun to find one common denominator in a growing list of chronic diseases: glycerol metabolism. Studies suggest that this process, an intermediate stage in the metabolism of carbohydrates and lipids, can play a part in everything from diabetes and obesity to infectious diseases and even aging.

Unfortunately, our understanding of the precise structure and function of some of the key players in glycerol metabolism falls short. In particular, monotopic proteins—proteins that are partially embedded on the membranes of cells but do not reach all the way down to the interior of cells—pose a challenge. Isolating and studying these macromolecules in their active, normal state—suspended in the semisolid environment of a membrane—is no easy task.

But last year, Associate Professor of Structural Biology Joanne Yeh established the first complete, three-dimensional structure of an enzyme that's common to all living things, and that's crucial to glycerol metabolism—Sn-glycerol-3-phosphate dehydrogenase, or GlpD, for short. The results of the study, which she completed with Research Assistant Professor Shoucheng Du and Research Associate Unmesh Chinte, made GlpD one of only a handful of monotopic-protein structures that have been determined to date.

“I think glycerol metabolism will be on the forefront of developing treatments for these diseases, and so many others,” Yeh says,

The first complete, 3-D structure of the Sn-glycerol-3-phosphate dehydrogenase (GlpD), an enzyme that's common to all living things and that's crucial to glycerol metabolism.

“since it is a pivotal yet underappreciated link among some very important metabolic pathways.”

Yeh's team completed their study in three months—far faster than usual—thanks to processes Yeh has fine-tuned over several years. In 2005, she published a paper in *Biochemistry* on novel peptide-based detergents—“peptergents,” as she calls them—that she developed to keep enzymes up and running outside of the membrane and to crystallize them.

Yeh specializes in X-ray crystallography, a technique in which a crystal scatters a beam of X-rays into many smaller ones. The angles and intensities of the beams are used to create a three-dimensional picture of the atoms and their bonds within the crystal.

Most of the study was completed in Pitt's X-ray crystallography facility, which Yeh directs. However, to obtain the best quality data, the team also used a cyclic particle accelerator, or synchrotron, at Argonne National Laboratory in Illinois and another synchrotron at Paul Scherrer Institute in Switzerland. Because time at such specialized facilities is so precious, Yeh's team labored together in Chicago around the clock. In Switzerland, Yeh put in 48 consecutive hours, all solo.

“It's very focused work,” she says, recalling the blur of exhaustion.

Finally, Yeh's team ran their data through advanced computations. After several months, the three-dimensional structure of GlpD emerged, down to the last atom. To make triple-sure their results were solid, Yeh's team solved the structures of GlpD in various states of producing a molecule that is important for cellular energy and fat production. The additional effort gave breadth and depth to their

understanding of GlpD's interaction with the membrane as well as its role in processes related to metabolism and energy production.

In March, Yeh's findings were published in *Proceedings of the National Academy of Sciences*. The paper also won an “Exceptional” rating on Faculty of 1000 Biology, a Web site in which top science faculty across the country highlight noteworthy publications. The rating was the cherry on top for Yeh. “It's really a nice validation of the significance of our findings and recognizes our research results as relevant to not only the structural biology community, but also other scientific disciplines,” she says.

Now, Yeh's lab is teaming up with MDs to make structural studies of human enzymes—specifically, those implicated in cardiac arrest and in metabolic diseases. She's leaving the possibilities wide open, though. After all, the list of opportunities is, well, Yeh long. ■

NANO NANO

In October 2007, the journal *Nanomedicine* published Yeh's two-part review of recent developments in nanobiosensors, super-small probes that utilize both biological and electronic features. “Integrating precise atomic, 3-D structure information can harness the innate specificity and sensitivity of biological systems,” she says. “By utilizing structural information, we have produced ultrasensitive biosensors that can be used in medical diagnostic applications. Our goal is to detect abnormalities in cells much earlier than what is currently possible.” —EV

PUTTING THEM BACK TOGETHER AGAIN

SCIENTISTS AIM TO MAKE WOUNDED SOLDIERS WHOLE | BY REID R. FRAZIER

Three years ago, Colonel Bob Vandre, then head of the army's Combat Casualty Care Research Program, was in St. Petersburg, Fla., for a U.S. Department of Defense conference on trauma care. Combat casualty care is a “bench to battlefield” discipline, concerned with making medical advances that can have an immediate impact on healing wounded soldiers. Vandre, an imaging physicist and dentist, had helped to create a handheld dental X-ray machine that comes in a padded, waterproof carrying case. He'd been talking with scientists in regenerative medicine and had even collaborated on research with the Pittsburgh Tissue Engineering Initiative, but he was cautious regarding the field's immediate prospects. “I thought it was all tissue cultures, and maybe 50 years from now we'd be able to do something with it.”

Body armor, shorter evacuation times, and improved battlefield medicine have saved thousands of injured U.S. troops in Iraq and Afghanistan. But they come home missing limbs. They are burned, scarred, and otherwise disfigured. The injured-to-killed ratio in all American wars, from the Revolution to Gulf War I, was 2.5 to 1. In Afghanistan and Iraq, that number is around 9 to 1.

So Vandre's jaw nearly hit the floor when he heard a talk at the Florida conference about recent advances in regenerative medicine, particularly lab-grown, transplantable human bladders. The field was much closer to helping wounded soldiers than Vandre had imagined. “This [treatment] isn't 50 years from now,” he remembers thinking. “It's now.”

Vandre spent two years drumming up support in Washington. He pulled together \$42.5 million to fund the newly minted Armed

Forces Institute of Regenerative Medicine, or AFIRM (It's supposed to be pronounced “affirm,” but everyone calls it “A-Firm,” Vandre says.) The White House promptly doubled the budget. And the Pentagon funded two consortiums—one led by the Pitt-UPMC McGowan Institute for Regenerative Medicine with Wake Forest University Baptist Medical Center—to pursue therapies of interest to the military.

The research tackles a wide range of regenerative medicine applications: soft tissue patches cultivated from pig bladders, a cement paste that regrows bone, hand transplantation, a nerve starter tube seeded with stem cells. Many of the therapies mimic processes already found in nature, says Alan Russell, director of the McGowan Institute and a University Professor of Surgery. “The salamander can regrow its heart—if you cut part of the heart out, it will regrow. Why does it happen in a salamander and not in a human—and could it?” says Russell, co-director of the Pitt-Wake Forest consortium.

“People ask, ‘How can you grow a new limb?’ Well, we did it once before—we did it in the womb. If we can understand a lot more about the biological signals that happened then and happen in animals like salamanders and newts, we can begin the process of creating these kinds of therapies.”

To that end, Professor of Surgery Stephen Badylak aims to regrow limbs and digits with biomaterials extracted from pig bladders. The tissue is washed of all cells, leaving behind the extracellular matrix—a honeycomb-like scaffold that attracts stem cells. Badylak hopes army medics can one day suture the material to a severe wound—a shattered arm, a sheared leg—to spur tissue growth and prevent amputation. The key, Badylak says, is getting the



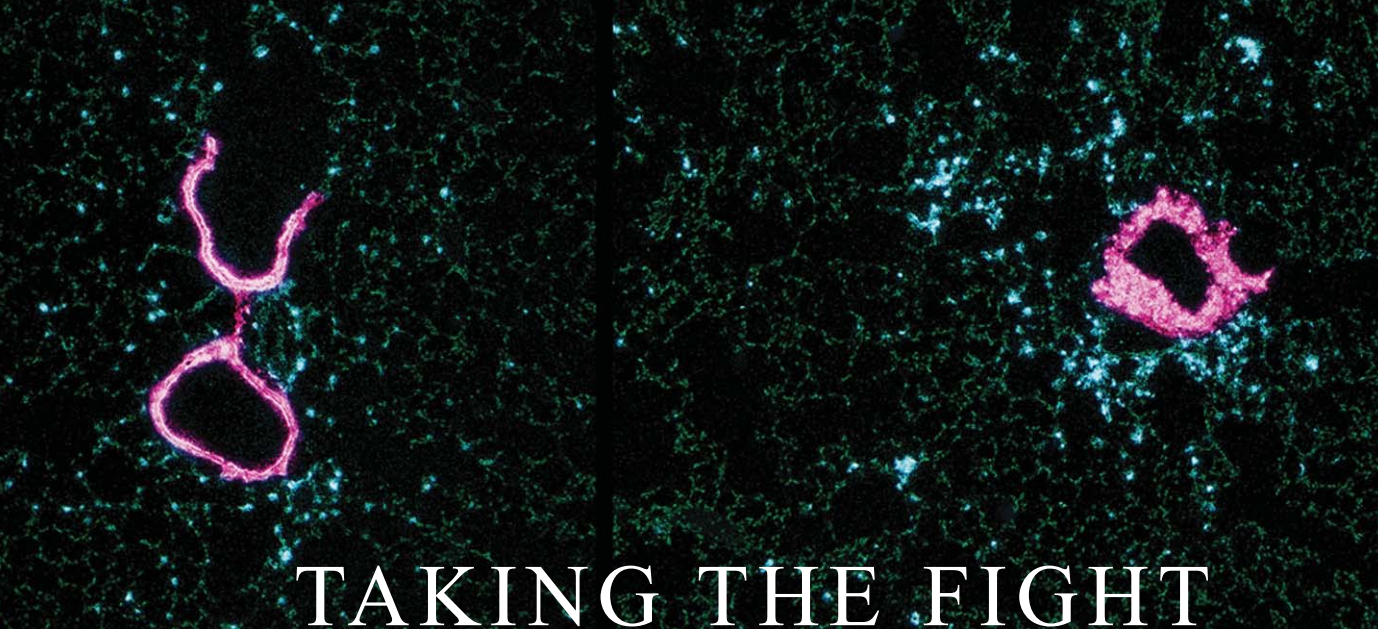
Speedy transport of injured soldiers has drastically increased survival rates in modern warfare, increasing demand for effective wound-healing technology.

signaling right. Normally, cells around a wound tell each other to scar. “We're trying to get the tissue to think, ‘I'm not injured, I just need to grow more tissue.’”

There are more than a dozen other projects at Pitt. Kacey Marra, a polymer chemist and assistant professor of surgery, is working on a tube that will train severed nerves to regrow. A craniofacial team involving Charles Sfeir, Prashant Kumta, and Elia Beniash from Pitt's School of Dental Medicine is exploring a calcium phosphate powder that can be mixed with any liquid—water, saline, even blood—and daubed onto an exposed wound to regrow bone. The substance would behave a lot like Plaster of Paris and could even be applied with a finger. William Wagner, a professor of surgery, is studying a biocompatible patch to treat compartment syndrome, caused when injury-induced inflammation—say, in biceps shredded by shrapnel—causes enough pressure that blood vessels constrict and the tissue dies. Surgical incisions release the pressure. Wagner's patch, seeded with stem cells or special growth proteins, can be sewn directly onto the incised compartment and stimulate regrowth.

It all sounds ambitious, but that's the point, says Badylak, who likens AFIRM to the Manhattan Project or the Apollo missions. “This is not just an incremental advance in a particular disease problem,” he says. “This is true tissue regeneration, replicating what you do as a fetus. Everything we've been taught ever since we started going to school is that human beings cannot regenerate limbs—it's a fact. So the first thing we've got to do is to get over that mindset.” ■

COURTESY U.S. ARMY



IL-22 regulates genes that help make antimicrobial proteins, key to fighting bacterial pneumonia, in airway epithelia. Here, we see two such genes, in pink, after being enhanced with IL-22 treatment.

TAKING THE FIGHT

TO PNEUMONIA

A NOVEL TREATMENT EMERGES FROM
A NEW CELL LINE | BY JOE MIKSCH

Worldwide, pneumonia kills more than 2 million children annually. Nothing kills more kids—not even AIDS and malaria combined.

In 2007, Jay Kolls, the Neils K. Jerne Professor of Pediatrics and Immunology in the University of Pittsburgh School of Medicine, discovered a protein target that may lead to therapies to treat bacterial pneumonia and play a vital role in creating a vaccine to prevent the disease.

Kolls, an MD and chief of the Division of Pediatric Pulmonary Medicine, Allergy, and Immunology at Children's Hospital of Pittsburgh of UPMC, says that the first step on the path toward rendering pneumonia impotent came in 1993 with the discovery of interleukin-17 (IL-17A), which is produced by a novel line of cells called T Helper Type 17 (Th17).

"It was found that [IL-17A] regulated neutrophil responses, and we knew that neutrophils [a type of white blood cell that devours pathogens] were critical for host defense against certain types of pneumonia," Kolls says.

This discovery gave birth to the idea that the Th17 pathway could be important to the immune system's defense of cells under assault from the bacteria that cause pneumonia.

Th17 produces a handful of cytokines—proteins that communicate with immune

cells, telling them to go out and take the fight to invading pathogens. IL-17A is involved in regulating neutrophil growth and recruitment, calling the cells into the lung from their perch in blood vessels nearby and, if necessary, ordering up more from the bone marrow.

"A neutrophil only lives eight to 10 hours, so when the lung sustains an infection that lasts longer than that, a signal is sent to the marrow saying, 'I need more troops,'" Kolls says.

Another of these Th17-related cytokines, interleukin-22 (IL-22), was of particular interest to Kolls—its production ramped up concurrently with IL-17A but appeared to serve a slightly different purpose. In 2006, Kolls read a study showing that IL-22 regulates antimicrobial peptides in the skin. The scientist wondered whether IL-22 did the same thing, immunity-wise, in other organs, particularly the lung.

The answer was yes. Examining the immune systems of mice infected with *Mycobacterium tuberculosis*, Kolls found that IL-22 activates a gene called lypocalin-2, which increases the population of antimicrobial peptides that attack invading bacteria. Many bacteria, including *M. tuberculosis*, scavenge iron from the body in order to survive.

"What lypocalin does is actually steal the iron back from the bacteria," Kolls says. "It's not effective against all bacteria, but it's certainly effective against this one."

"[IL-17A and IL-22] don't have completely overlapping worlds, but they do both have roles in regulating immunity in the lung," Kolls notes. IL-22's world, though it works in synergy with IL-17A's, might be a bit more important. Mice deficient in IL-17A succumbed to infection in about 48 hours. Those deficient in IL-22 "had trouble within as early as 24 hours," Kolls says.

By increasing the level of IL-22 in the mice's lung tissue, Kolls was able to cure the rodents. More IL-22, Kolls found, meant that there were more iron-stealing proteins and progressively more resilient lung epithelial cells, which could better handle the insults and injuries caused by the pneumonia bacteria.

Kolls says he thinks the process will work the same way in humans. Doses of recombinant IL-22 could be used as a prophylactic treatment against tuberculosis, he says, priming the immune system for a fight.

It will be as much as a decade before IL-22-related therapies are approved to treat or prevent tuberculosis in people, but Kolls' work presages a treatment more effective than antibiotics that comes without the risk of creating antibiotic resistance.

"We could use it as a prophylactic regimen; it could be used as a vaccine," Kolls says. "There's a 5- to 10-year timeline before this could probably happen, but we've learned a lot, and we're making progress." ■

COURTESY GREGORY KOLLS