

ISSUE

02

FALL
2017

Seek

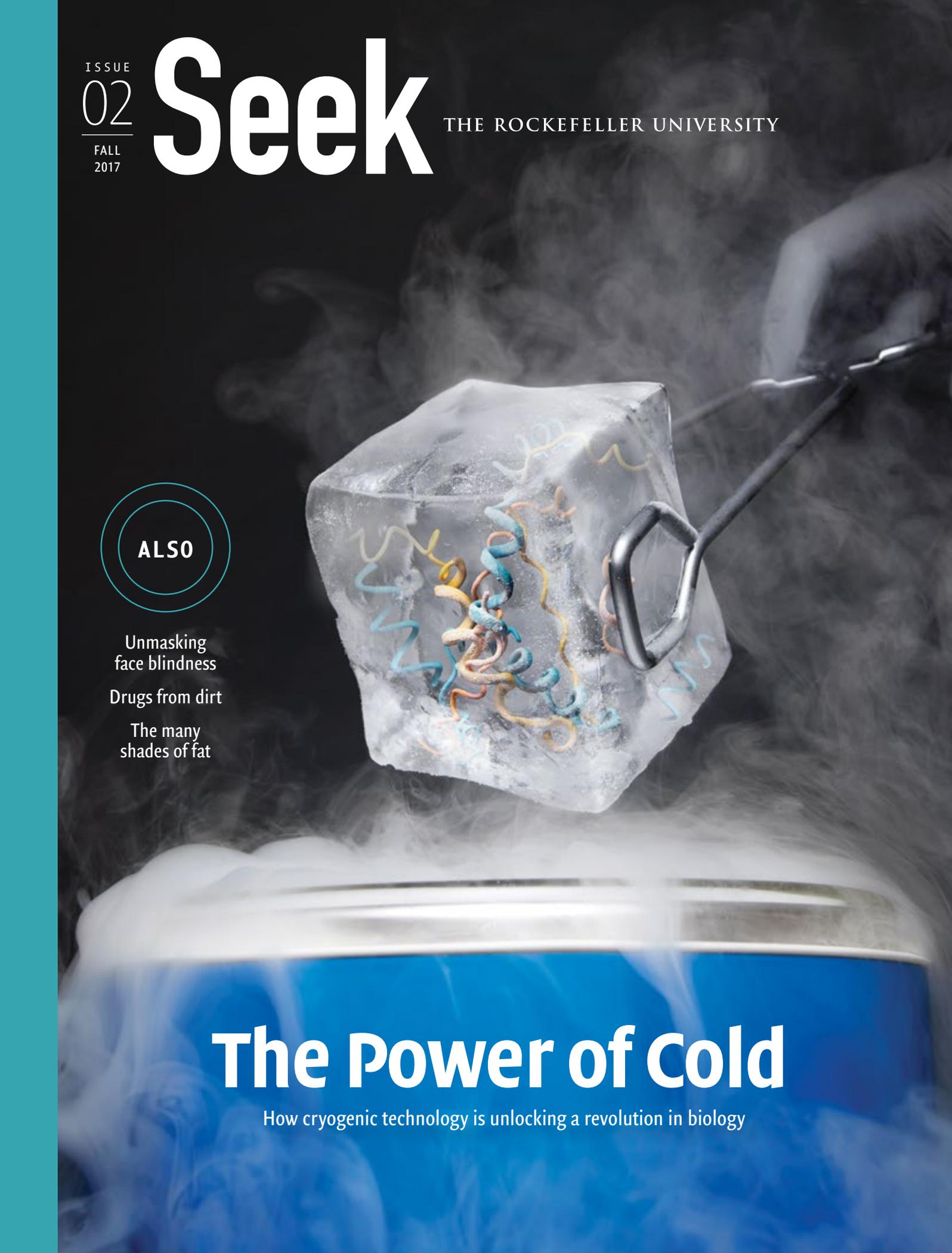
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ALSO

Unmasking
face blindness

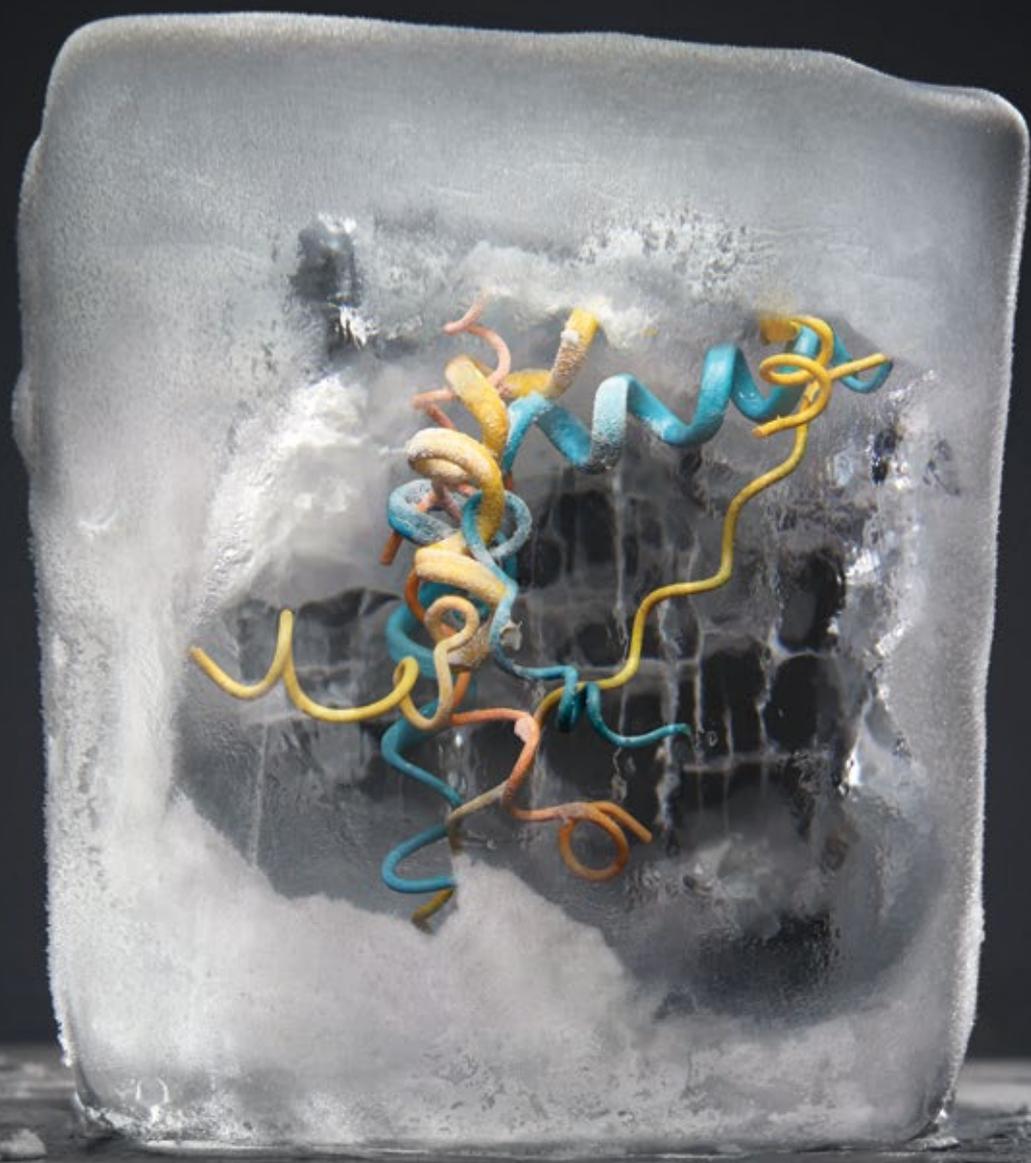
Drugs from dirt

The many
shades of fat



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Michael W. Young

The more he looked, the prettier the science became. Young's work on the biological clock led to trailblazing discoveries of genes that make all organisms tick—and to a Nobel Prize.

“When the time comes, evolution will be ready.”

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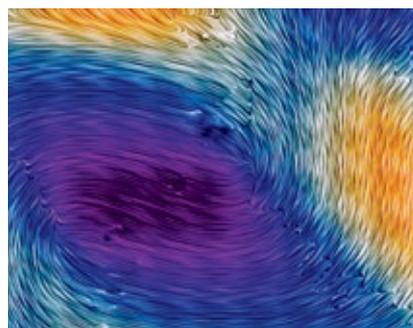
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Scientists are looking 11 million years back in time for clues to conquer HIV. They're also disassembling the brain's internal compass and **modeling microbes' motion patterns.**



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Seek

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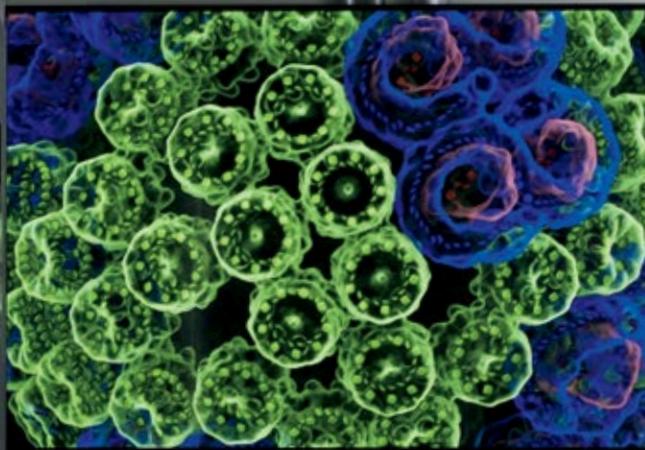
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Processing power Discovery is getting data-heavy. With new tools like cryo-electron microscopy and whole-genome sequencing, even simple experiments can generate many terabytes of raw information. Rockefeller's new high-performance computing cluster gives labs access to processing infrastructure capable of some 57 trillion calculations per second. It's designed for the next phase of science, a future where scientists can find meaning among an unlimited number of zeros and ones.

PHOTO BY MARIO MORGADO

Reported by Katherine Fenz,
Eva Kiesler, Wynne Parry, Zachary
Veilleux, and Sarah Williams.

FOREFRONT



TIME MACHINE

Paleontology on a (very) small scale

OUR PREHISTORIC PAST is a blur. Scientists are not sure, for example, exactly where the first *Homo sapiens* emerged around 200,000 years ago, or why our cousins, the Neanderthals, went extinct more than 150,000 years later.

On the other hand, they do know quite a bit about our immune system's historic battles, dating as far back as 11 million years. In studying DNA of orangutans, macaques, and other present-day primates, Rockefeller biologists have revealed how our hominoid ancestors conquered a retrovirus—a pathogen of the same class as modern HIV. This fresh DNA contains ancient traces of infection: when our predecessors contracted the retrovirus, their genomes were sprinkled with clippings of its genetic material. In some cases, the infected cell happened to be a sperm or egg cell, and the imprint got passed



DATA

The human lineage and the gorillas' parted ways around **10 million** years ago.

on for generations, creating a genetic fossil record.

“Analyzing viral fossils can give us a wealth of insight into events that occurred in our distant past,” says Paul Bieniasz, who led the research, published in *eLife* in April. Working with scientists at the University of Glasgow, his lab has compiled a near-complete inventory of such relics within the genomes of old-world monkeys and apes, and used it to investigate the warfare between primates and their microbial enemies.

In pinpointing the precise molecular maneuvers that eventually helped our ancestors get the retrovirus out of their systems, Bieniasz and his colleagues have learned about its weak spots—insights he says could potentially be harnessed to combat modern retroviruses, including HIV. ☉

Like us, dolphins share duties, speak in dialects, and mourn their dead.



Foster, a ten-year-old Atlantic bottlenose dolphin, was one of the first to interact with an underwater touchscreen.

EXTRA-HUMAN INTELLIGENCE

Screen time in the aquarium

WE KNOW DOLPHINS are smart, but will they use an iPad? Yes, scientists have discovered: with an underwater touchscreen built especially for them, and a little bit of training, our aquatic peers will even enjoy a game of Whack-a-Mole.

Rockefeller scientists, collaborating with colleagues at Hunter College and the National Aquarium in Baltimore, have used advanced optical technology to develop the first touchscreen computer through which dolphins

can interact. Neurophysiologist Marcello O. Magnasco, who led the work, says the device allows dolphins to make deliberate choices, and can be used to study certain aspects of dolphin intelligence, including the animals' vocal learning behavior and their ability to communicate with symbols.

“It has always been hard for humans to keep up with dolphins,” Magnasco says, “but this system will help us follow them in any direction they take us.” ☉



BEYOND BORDERS

Magic bullets in the blood

SOON THREE YEARS WILL have passed since the current Zika epidemic broke out in Brazil. During this time, expectant parents on several continents have been at the mercy of insect sprays and nets, and will

Zika-tainted blood samples were collected from residents of Pao da Lima, Brazil (above) and Santa María Mixtequilla, Mexico.

remain so for the time being. There are no treatments or means of prevention for this mosquito-borne disease, which causes brain defects in children born from infected moms.

But scientists now have fresh optimism, incited by a discovery reported in *Cell* in May. In blood samples from Zika-infected donors, they've found what could become a potent weapon against the disease: antibodies that can neutralize the virus. And they have already initiated work to develop Zika-prevention drugs based on these antibodies—work that's being led by Davide F. Robbiani, a research associate professor in Michel C. Nussenzweig's lab. ☉

DATA

97

Number of countries where people are at risk of Zika infection.

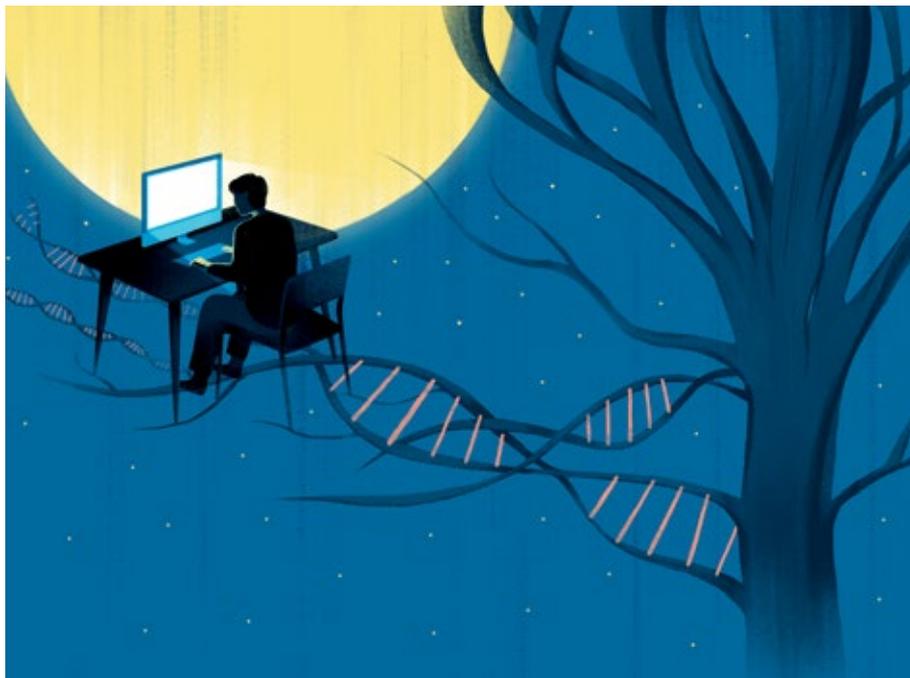
DAILY RHYTHMS

The gene that keeps you up at night

AT 11 P.M., there's barely enough time to get a good night's sleep before the alarm goes off. Also, there's Netflix, online shopping, and e-mail.

It turns out that many of us are biologically destined to be night owls. Scientists have discovered a common mutation that slows people's circadian rhythm, the molecular clock that helps the body know what time it is, causing them to stay alert way past their supposed bedtimes.

The findings were reported in *Cell* in April by research associate Alina Patke and Michael W. Young, who won a Nobel Prize this year for his decades of work on sleep-wake cycles (see "Michael W. Young," page 48). Collaborating with a sleep clinic, Patke and Young discovered the mutation in a patient whose internal clock was running late, a condition known as delayed sleep phase disorder. In scouring public databases, they later identified close to



30 people with defects in the same gene, called *CRY1*—and sure enough, all turned out to have the disorder.

Night owls won't necessarily benefit from knowing they have the *CRY1* mutation, but the researchers say its discovery may advance research on sleep disorders, and maybe lead to therapies. ☉

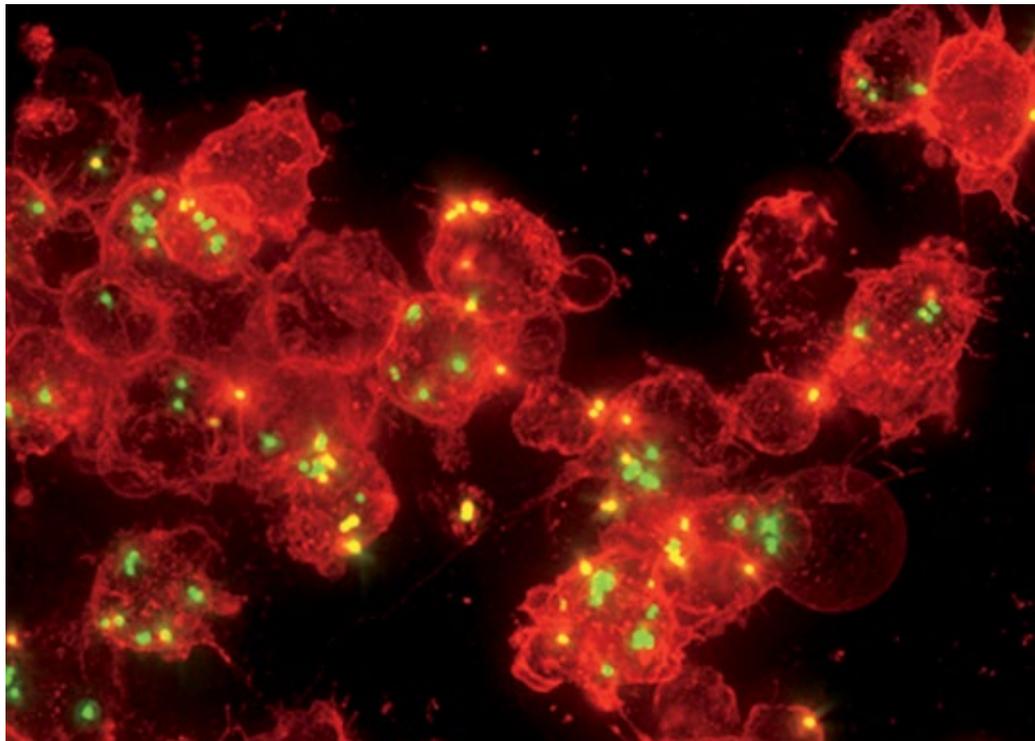
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90,000

Number of Americans
infected with drug-resistant
Staph superbugs each year.

60 percent of a
bacterium's cell wall
consists of carbohydrates.

Large immune cells, shown in red, have been summoned by lysibodies to engulf *Staphylococcus aureus* bacteria (green dots).



DRUG INCUBATOR

Half-human, half-viral: A hybrid weapon against dangerous microbes

HOSTILE GERMS are everywhere—in airplanes, on door-knobs, in our stomachs—and they may spread unpleasant diseases like salmonella, tuberculosis, or worse. Yet most of us have no reason to panic. The immune system is keeping us relatively safe.

The system has room for improvement, however: every once in a while, an unwelcome pathogen will let itself in. Among its flaws is the inability to spot molecules called carbohydrates, which bacteria surround themselves with. If it weren't for this blind spot, the immune system would have more ways to catch unwanted trespassers: a bacterium dressed up in certain carbs

would be parading a bull's eye before it.

Rockefeller scientists have engineered a molecular hybrid they hope will give the immune system some pointers, directing it toward dangerous bacteria it may not otherwise see. The agent, called a lysibody, is a cross between a viral molecule and a human one.

Some viruses that prey upon bacteria produce enzymes that bind tightly to specific carbs in a bacterium's outer shell. In creating the lysibody, Vincent A. Fischetti and his team borrowed this function from a virus; the molecule's lysin part latches on to the invading pathogen while its human part activates the immune system.

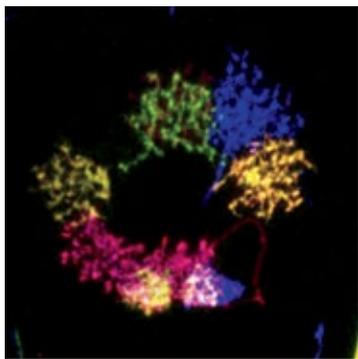
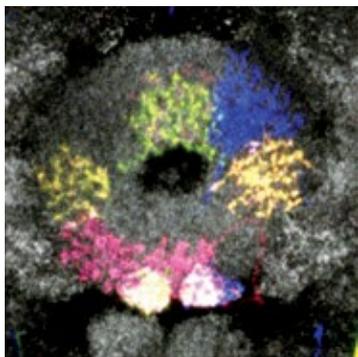
The researchers have successfully used lysibodies to treat mice infected with *Staphylococcus aureus* "superbugs" that antibiotics cannot beat, and they are now planning clinical trials to find out if these molecules could be used to treat similar infections in people.

"The approach could make it possible to develop a new class of immune-boosting therapies," says Fischetti—therapies that potentially could be used against any disease-causing pathogen, be it a bacterium, virus, parasite, or fungus. ☉

Shortest way to the fruit tray? Fly straight, turn left, and dive.

ALL ANIMALS HAVE an internal compass. If you close your eyes and turn around, you'll probably still have a sense—without looking—of which direction you're facing. Even the humble fruit fly excels at this test, making it a neat tool for scientists who study how the brain navigates.

In experiments described in *Nature* in May, Gaby Maimon and his colleagues challenged flies with direction-finding exercises while imaging their brain cells, leading them to discover a group of neurons tasked with updating a fly's compass as it turns. "Our findings may have relevance for understanding spatial cognition in larger brains—including, perhaps, our own," Maimon says. ©



Groups of neurons in a fly's brain (colors) compute the direction of its head as it moves.

The secret code of aerial particles



Volunteers sniffed vials containing everything from vanillin to stinky-cheese molecules.

SIGHTS AND SOUNDS are unfailingly predictable. The laws of optics ascertain that when a human eye meets light with a wavelength of 470 nanometers, it will see blue. Acoustics principles are equally precise: middle C will ring in any ear subjected to a 261.6 hertz sound wave.

What about smells?

In theory, they too could be forecast based on the chemical structures of molecules that waft into the nose. But currently, we don't know how to make such calculations; the only way to tell if a substance will smell like roses or turpentine—or to even know it has a smell—is to actually inhale it.

Scientists have taken the first steps toward making odors more calculable, however. Earlier this year, the neuroscience lab of Leslie B. Vosshall, Robin Chemers Neustein Professor, reported in *Science* that they had procured smell data on close to 500 molecules—the largest collection of

its kind—and used it to create a prediction algorithm.

It took a village. Close to 50 volunteers came to the lab to sniff the molecules and note their characteristics and intensities. The Rockefeller team then handed this data over to collaborators at IBM, who organized a crowdsourcing contest: computer scientists from around the world were asked to build models that associate the human data with chemistry parameters.

The resulting algorithm is a blend of several winning solutions. It needs more work, but the scientists hope it will eventually open a window into the poorly understood biology of olfaction, allowing them to study how brain signals get triggered when molecules interact with smell receptors. Thus far, the tool is the furthest anyone has come in computing smells—and it's already quite good at guessing some odors, like garlic and fish. ©



END OF AN ERA

David Rockefeller's passion for science—and small living things

FOR 75 YEARS, David Rockefeller was the leading force behind the institution that bears his family name. He was a keen and influential benefactor, a visionary leader, and a regular presence on campus. Although his death on March 20, at the age of 101, deprives the university of its most steadfast supporter, David's impact is indelible: his dedication to excellence has

David Rockefeller catching beetles.

“David’s efforts never looked strained. They were driven by curiosity and an intrinsic joy of learning.”

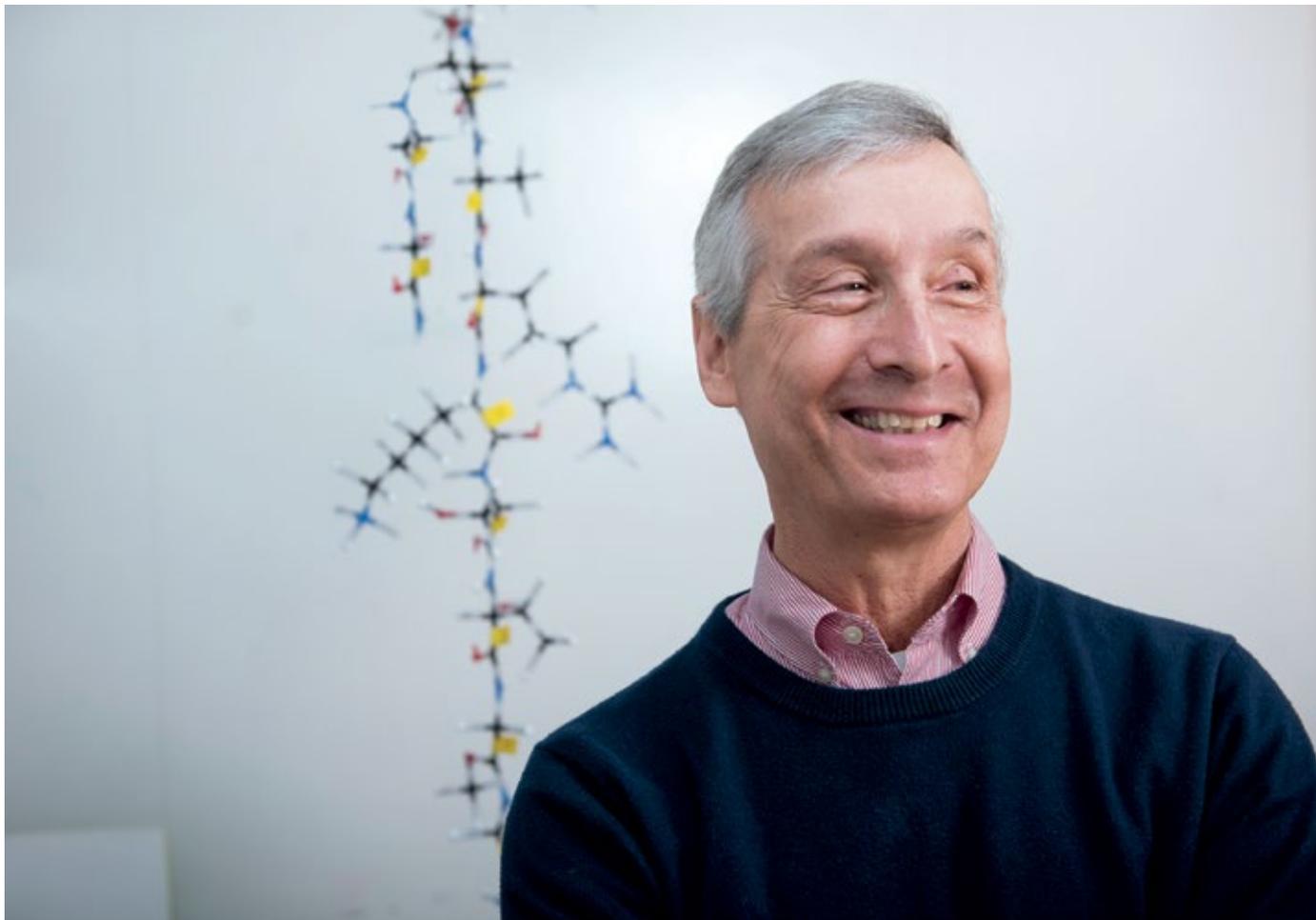
—Günter Blobel, Rockefeller professor and Nobel laureate

shaped several generations of Rockefeller scientists, and will reverberate across campus for decades to come.

In addition to being an astute businessman, a New York City developer, and a promoter of the visual arts, David was an amateur entomologist. In his spare time, he collected beetles. Among his bequests is a collection of 75,000 specimens, delicately filed inside custom-made cabinets. ©

Can cancer cells be rehabilitated?

With C. David Allis



MOST CANCER THERAPIES were developed on the premise that the body must be purged of disease—whether by cutting tumors out or by poisoning them with chemicals or radiation. But tomorrow’s treatments might be based on subtler, more aikido-like solutions.

One such approach is a new class of drugs that, rather than destroy tumors, aims to put cancerous cells on a path back to normalcy and let them lead healthy, productive lives. Such a feat could be possible thanks to epigenetics, a type of indexing system superimposed on DNA. The system exists in all animals and plants, and it ensures every gene gets used precisely the way it should—in the right cell, at the right time, to the right extent.

Allis has spent four decades studying epigenetic mechanisms.

C. David Allis, a pioneer in epigenetics, says that cancer is often the result of this indexing gone wrong. In many leukemias, for example, certain blood cells that ought to be keeping a low profile instead switch on genes that boost their own growth and survival. In recent work published in *Nature*, Allis, along with an international team of researchers, presented concepts for drugs that would work by resetting the cells’ internal programming. Their hope is that the approach will be more effective, and less prone to causing side effects, than existing cancer treatments.

We asked Allis, the Joy and Jack Fishman Professor, to tell us more about epigenetic therapy and its promise.

Help us understand what “epigenetic” means and how it’s relevant for cancer.

The word literally means “in addition to” DNA, and it refers to the fact that the DNA in our cells doesn’t exist in isolation, or act on its own. It’s tightly wrapped around spools of proteins called histones, which in fact do a lot more than package DNA—they function like master switches determining what sets of genes in a cell should be active or inactive.

Histones carry an intricate and vast repertoire of chemical marks, which are constantly being modified to turn the expression of genes up or down. In the past few years, we’ve learned that the enzymes responsible for creating or interpreting histone marks are surprisingly often mutated in cancer. In many cases, the disease is the result of both genetic and epigenetic errors acting in concert.

There is often not much we can do about classic genetic mutations in which a cell’s DNA sequence has been altered. It’s hard to make drugs to restore those changes. But epigenetic abnormalities are fixable, and potentially reversible, at least on paper. There are now several compounds that target different classes of histone-modifying enzymes, and some are showing promising results in clinical trials.

What can you tell us about the leukemia drug your lab is developing?

Liling Wan, a postdoc in my lab, has discovered that a protein called ENL, together with other proteins, acts as an engine for tumor growth in leukemia cells. ENL is an epigenetic “reader”—its job is to recognize specific marks on histones and activate genes accordingly. More than 35 percent of people with acute myeloid leukemia—and about 70 percent of infants with the disease—have a flawed version of this protein in their blood cells, leading to the faulty activation of cancer-promoting genes.

With support from the Tri-Institutional Therapeutics Discovery Institute, we are now working to develop a compound that can prevent this disease mechanism.

Is the drug working?

We have a lot more work to do before we can make an effective ENL inhibitor and test it in patients. That said, we’re very encouraged by a series of experiments we did in leukemia mouse models. When human AML cells are transplanted into mice, the animals get cancer. But if we remove ENL from these cells by genetic engineering, the cells don’t divide as quickly, and the animals live longer.

“Now that the field is a hotbed for drug discovery, it has taken on a whole new dimension.”



DATA

Uncoiled from histones and stretched out, a cell’s DNA would be about **2 meters** long.

Another interesting observation came from work with an experimental leukemia drug that targets Bet, an epigenetic enzyme of the same class as ENL. In our mouse model, we were able to boost the effectiveness of this Bet inhibitor by removing ENL. These findings strengthen our confidence that a well-designed ENL inhibitor could be effective—either by itself or in combination with Bet inhibitors.

Could this potentially work on cancers other than leukemia?

Yes, and that’s a possibility we’re currently exploring. It turns out that many children with Wilms’ tumor, a rare and often deadly form of kidney cancer, have mutations affecting ENL, leading to the faulty expression of tumor-causing genes similar to those we see in leukemia. Liling and others are now doing experiments on patient-derived Wilms’ tumor cells to see if and how their mutations are linked to epigenetic function.

You started to work on histones when few other scientists were interested, and long before epigenetics became a buzzword. How has the field changed?

In the early 1980s, when my lab first started out, we didn’t even know why histones are chemically modified. It was a hypothesis, originally proposed by Rockefeller biologist Vincent Allfrey in the mid-1960s. And chromatin, the amalgam of DNA and histones, was not nearly as fashionable a topic as it is today. Still, we were possessed to think that both histone-modifying enzymes and the histones themselves are biologically important, seeing that all organisms have them.

It took several decades before we and others began to realize that epigenetic changes can cause cancer, developmental defects, and many other diseases—and before we discovered that these changes are therapeutically pliable. Today, chromatin biology and epigenetics are informing essentially every aspect of biology. Now that the field is a hotbed for drug discovery, it has taken on a whole new dimension.

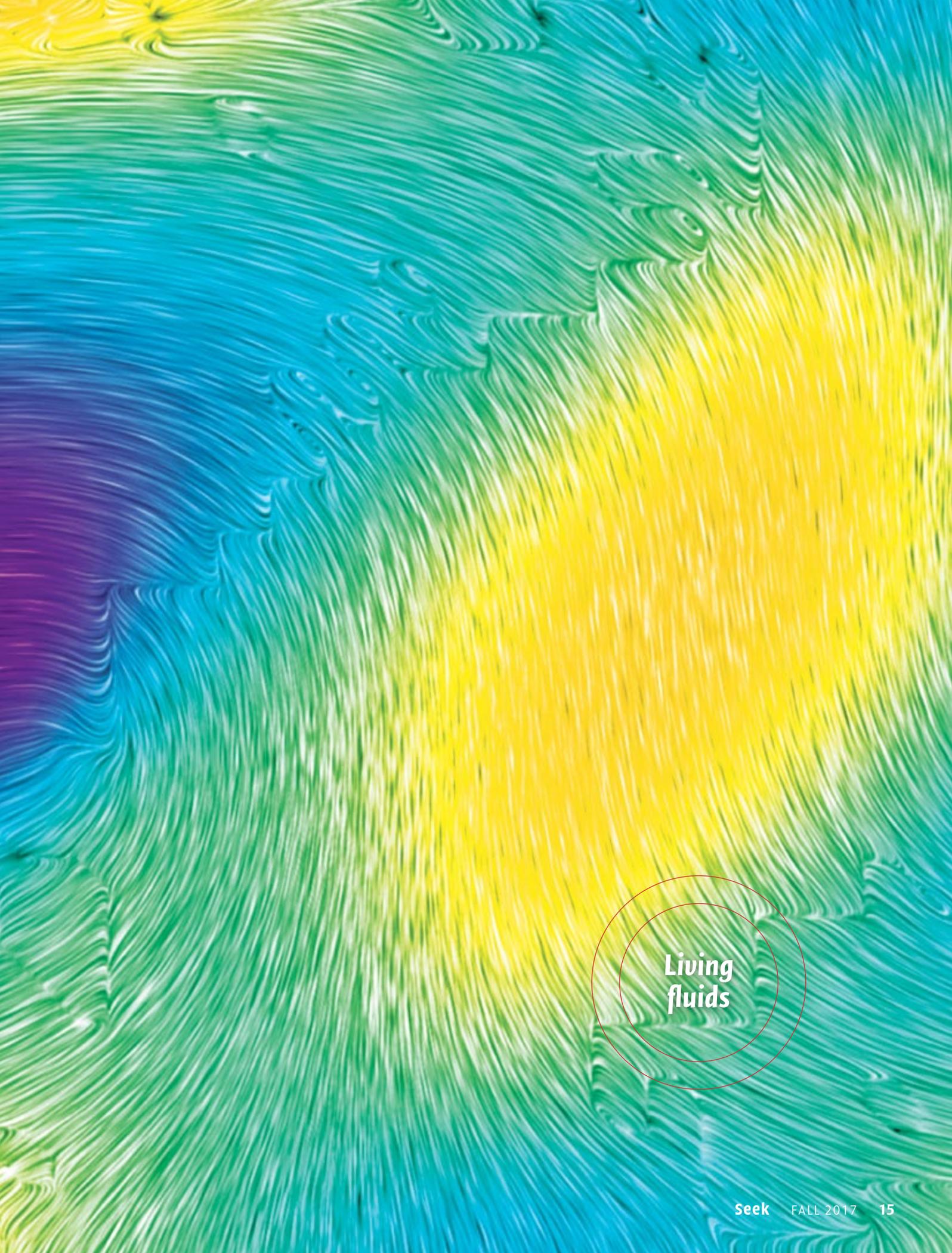
It’s an incredibly exciting development to be part of. ©

SNAPSHOT

Fleeting beauty

SWIMMING BACTERIA produce hypnotic vortex patterns, but only when they're moving with the right amount of vigor. Too fast, and the meticulous choreography will disintegrate into dull turbulence. Rockefeller fellow Tyler Shendruk, a physicist, discovered a mathematical signature that describes the shift from orderliness to chaos. In this image, his model simulates bacterial movement through a narrow conduit.

CENTER FOR STUDIES IN PHYSICS AND BIOLOGY / THE ROCKEFELLER UNIVERSITY



Living
fluids



How can medical advances be extended to those who need them most? An infectious disease doctor adds molecular virology and social psychology to her repertoire.

Chibuzo Enemchukwu

By Alexandra MacWade

SHE IS COMFORTABLE with long silences. “It’s okay to sit and wait,” she says. And if she breaks the silence, it may seem like she’s just changing the subject. But it is in these moments, when she gently guides the conversation elsewhere, that Chibuzo Enemchukwu often reaches a diagnosis. With her, questions like “What are your symptoms?” and “How long have you had them?” aren’t as important as: “Where are you from?” “What do you enjoy doing?” and “Tell me about your family.”

Enemchukwu, an infectious disease doctor, has always been fascinated by people and driven to do work that helps others. As a fellow in Rockefeller’s Clinical Scholars Program, she splits her time between twin pursuits at two locations: In virologist Charles M. Rice’s lab at Rockefeller, she’s working to understand why certain people develop severe reactions to the yellow fever vaccine. In Sarit Golub’s lab at Hunter College, a few blocks away, she conducts social psychology research, seeking to improve HIV prevention within sexual, gender, and ethnic minority communities.

Merging virology and social psychology is unusual, but it doesn’t seem strange to Enemchukwu, who was a psychology major at Duke University. As an undergraduate, she thought she might become a psychiatrist until her coursework nudged her in different directions—she took courses in social medicine that got her interested in health disparities across ethnic communities, and was then drawn to infectious disease. Both topics eventually inspired her to earn her medical degree, from the University of North Carolina.

And in fact, Enemchukwu’s current projects are not as different as they may seem. Fundamentally, both ask how a patient’s characteristics—genetic or social—can lead to vastly different outcomes. In the context of yellow fever, Enemchukwu seeks to understand why in rare cases, a person may develop a life-threatening reaction to the vaccine. And in her HIV project, she focuses on a disease-prevention strategy called pre-exposure prophylaxis, or PrEP, trying to get to the bottom of why its prescription and use is subject to racial and ethnic disparities.

“Ultimately, we are asking the same question,” she says. “How does who you are affect your health?”

SINCE IT BECAME available five years ago, PrEP has been prescribed to tens of thousands of Americans at risk of contracting HIV. A pill sold under the brand name Truvada, it stops the virus from replicating and establishing itself in the body. When used correctly—it must be taken daily, and patients need to see their doctor for follow-ups—it is more than 90 percent effective.

Yet even as more and more prescriptions are being filled, health experts worry that the drug is not living up to its promise. Data shows that black people fill as little as 10 to 12 percent of all Truvada prescriptions, despite the fact that 44 percent of people receiving their first HIV diagnosis are black. Within both black and white communities, most new HIV diagnoses occur in men who have sex with other men (many but not all of whom identify as gay or as belonging to another sexual minority group).

“What we’re seeing is that the people

“I want to take evidence and learn how to turn it into action. Here, the root of that idea is in every lab.”

who need PrEP the most aren't on it,” Enemchukwu says. “We have an HIV epidemic that disproportionately affects black men who have sex with other men, and we have this great drug that these populations aren't taking.”

A simple answer would be that black men who have sex with men don't want PrEP. But that's not usually the case, Enemchukwu has learned: when these men are offered the drug, most are in fact interested in taking it. Instead, she and others have found that stigma plays a big role, as does the fact that those who would benefit most from the regimen have limited access to it, due to lower rates of health insurance coverage or difficulty accessing the healthcare system in general.

“HIV stigma is real, as is gay stigma, particularly in black communities,” Enemchukwu says. “Studies have shown that black patients are often more reluctant to disclose they're gay. If you're not 'out' in that sense, you won't be going to an LGBTQ center, and that's historically where the drug has been prescribed the most.” Many community-based primary care clinics—which some ethnic minorities are more likely to rely on—lack either the resources or the expertise to provide it.

In researching PrEP use, Enemchukwu has learned that there is also stigma and misinformation surrounding the medication itself, and in some cases it is being perpetuated by doctors. Evidence suggests that if you're a man who has sex with other men, your doctor will be less likely to prescribe PrEP if you're black than if you're white. Many assume that the reason rates of HIV infection are skyrocketing in some black communities is because black men tend to lead more promiscuous sex lives. But studies show the opposite: that high-risk black men have fewer sex partners and are more likely to use condoms than their white counterparts.

The research points to another factor: that your likelihood of encountering someone who's HIV-positive is simply higher if

you're a black man. “It's about probabilities and social networks,” Enemchukwu says.

In one of her projects, Enemchukwu is working with Golub and the New York City Department of Health to study PrEP implementation in the city's clinics. They are looking at ways to make the drug accessible to everyone who needs it, and to ensure that providers have the tools they need to prescribe it.

The most challenging roadblock, she says, is changing prevalent attitudes about who should and should not be on PrEP. “We have to find a way to convince both providers and the public that it's time to drop all preconceived notions about black gay men and PrEP users in general. Prescribing someone PrEP will not encourage irresponsible sexual behavior—it will simply offer an effective means of protection to the most vulnerable.”

ENEMCHUKWU WAS BORN in Boston and grew up in central Florida, near Orlando. Her parents are from the same town in Nigeria but didn't meet until they were both in Boston studying to be pharmacists. When Enemchukwu was young, they would take her and her five siblings to visit her grandparents in their home country. She was struck by how many people, not just her relatives, would come to her grandparents' house when they learned that family from America was visiting.

“We'd bring tons of suitcases with us, packed full of medicine, shoes, clothes to give away,” she says. The visitors would often stay to talk, finding comfort in telling Enemchukwu's family their problems.

Years after her childhood trips to Nigeria, Enemchukwu again found herself listening to similar stories. She took a leave of absence after her third year of medical school to join Karibu, a nonprofit organization based in Madrid that provides medical and other services to Sub-Saharan African immigrants. The work touched on a theme that interested her, particularly in light of her parents' immigration to the U.S.—her attention was rapt each time someone spoke about what it had been like to leave their home and go someplace else to make a better life. The conversations were often challenging, and there were vast cultural divides between her and some of her patients, not to mention language barriers. The experience turned out to be invaluable.

In medical school, Enemchukwu had learned the basics of bedside manner: she and her fellow students were taught not only to sit when they interacted with patients, but to sit lower than their patients to appear less intimidating. They were told to make eye contact, and they learned how to clearly explain diagnoses and complicated procedures. From her sunken chair, Enemchukwu took great care to make eye contact with the immigrant patients she met in Madrid, but she also began developing her own style. For starters, she allowed long pauses to elapse in the conversation, and she figured out the kinds of questions she should be asking.

Many of the people she saw at Karibu complained of pain: back pain, shoulder pain, headaches. “I came to realize that many of these symptoms were physical manifestations of depression and stress,” she says, “which is not unusual in immigrant communities.” Her questions turned to the more essential: “Where are you sleeping at night?” “Are you getting enough food to eat?” and “Do you have work?”



With Charles M. Rice as her mentor, Enemchukwu is studying a rare, life-threatening reaction to the yellow fever vaccine.

campaign, Enemchukwu has helped acquire tissue samples from viscerotropic disease patients. The lab is now trying to pinpoint what genes may be driving the condition and how the vaccine's viral component interacts with an infected person's cells.

Enemchukwu is beginning to understand the amount of meticulous work it takes to move basic science projects forward. "Working in the clinic, I didn't fully appreciate all the challenges involved in getting new drugs to patients," she says. Rice himself can attest to the Sisyphean aspect of the effort: it took him 30 years to replicate the virus that causes hepatitis C, a discovery that ultimately led to a cure for that disease.

"There are several barriers with this project, too," Enemchukwu says, "including finding the tissue samples, which are extremely hard to come by. Once we have them, we hope that our experiments will work. And once we get the results, we hope they will put us on the right track."

Hervision is that this work will help create methods to screen people with an increased risk for developing severe reactions—including the elderly, infants, and those with certain autoimmune diseases—before they are vaccinated against yellow fever.

ENEMCHUKWU IS A little more than a third of the way through the Clinical Scholars Program, and for the first time in her life, she doesn't have a clear idea of what her next steps will be. "It's both distressing and refreshing," she says. She knows that she would like to start a research career that combines her interests in infectious diseases, minority and immigrant populations, and health disparities. And she is interested in collaborating on global health projects and continuing her work on HIV.

"I also know that I want to see patients, and I want to teach medical students and residents," she says. "Those things I know. But other than that?" She takes a long pause and smiles. "That's TBD." ☺

Enemchukwu completed her fellowship in infectious disease at the Albert Einstein College of Medicine, and did a significant part of her clinical work at the Jacobi Medical Center in the Bronx, one of the poorest and most diverse places in the country and one greatly affected by HIV. At the end of her fellowship, she took another trip abroad, this time to Malawi for a month to help with a study on HIV and malaria. In the hospital where she worked, most of the adult patients she saw in the general wards had end-stage AIDS. It was emotionally overwhelming, and she was heartbroken to see how the modern healthcare she had been trained to deliver was glaringly absent from a part of the world where it was critically needed.

"The trip was absolutely life-changing," she says. "When I came back, I was motivated to do something. I had to do something." She thought about traveling to Malawi or other places for a few months every year while continuing to work as an infectious disease doctor in the U.S., but that "would have been putting a small Band-Aid on a gaping wound, and would really just have been a way of making myself feel better," she says. Instead, she applied to be a clinical scholar, and join a rigorous multiyear training program in which clinicians add to their medical degree a master's in clinical and translational research.

She liked that the program would prepare her for a career in patient-oriented research,

and that she could create her own curriculum. But there was more: "I don't want to do research for the sake of doing research," she says. "I want to take evidence and learn how to turn it into action. At Rockefeller, the root of that idea is in every lab."

BEFORE WORKING WITH Rice on a project to understand life-threatening reactions to the yellow fever vaccine, Enemchukwu hadn't set foot in a basic science lab. "I made a lot of mistakes running experiments," she says, "but I learned a lot."

Like PrEP, the yellow fever vaccine is highly effective. With one dose, most people will be fully protected against the virus for life. But Enemchukwu and Rice are focusing on a small fraction of patients—four out of every million—who will develop severe reactions to the vaccine. Most of them have neurotropic disease, an illness resembling brain inflammation, which is severe but not usually life threatening. Others develop viscerotropic disease, which is similar to the yellow fever disease itself, and devastating—these patients, who may suffer from liver or kidney dysfunction, bleeding, and fever, rarely survive.

The Rice lab has shown that these fatal reactions are likely caused by a defect in the immune system. Together with collaborators in Brazil, where there was recently a yellow fever epidemic and a mass vaccination



A great chill has unlocked biology.

Electron beams are bringing life's
best-kept secrets into focus.

By Alexander Gelfand

Photograph by The Voorhes

Thanks to ice, structural biology is on fire. With new ways to snap-freeze and visualize molecules mid-movement, scientists are churning out new knowledge about life and disease with unprecedented speed.

It looks like a fancy espresso maker,” says Mark Ebrahim, gesturing towards a finely machined assembly of metal components housed in the basement of the Collaborative Research Center.

But Ebrahim is no barista. Rather, he is a physicist. And as the safety sticker on it suggests (“Warning: Cold Surface”), the device before him is no coffee maker.

Instead, it is a high-powered electron microscope that pumps focused beams of charged particles through protein samples that have been flash-frozen in liquid ethane, and then kept at a chilly -180°C by the contents of huge liquid-nitrogen tanks. Super-sensitive cameras record the electrons that pass through the samples, and powerful software algorithms use the resulting data to generate three-dimensional images of the proteins at near-atomic resolution. (Ebrahim, a senior staff scientist, keeps the equipment running.)

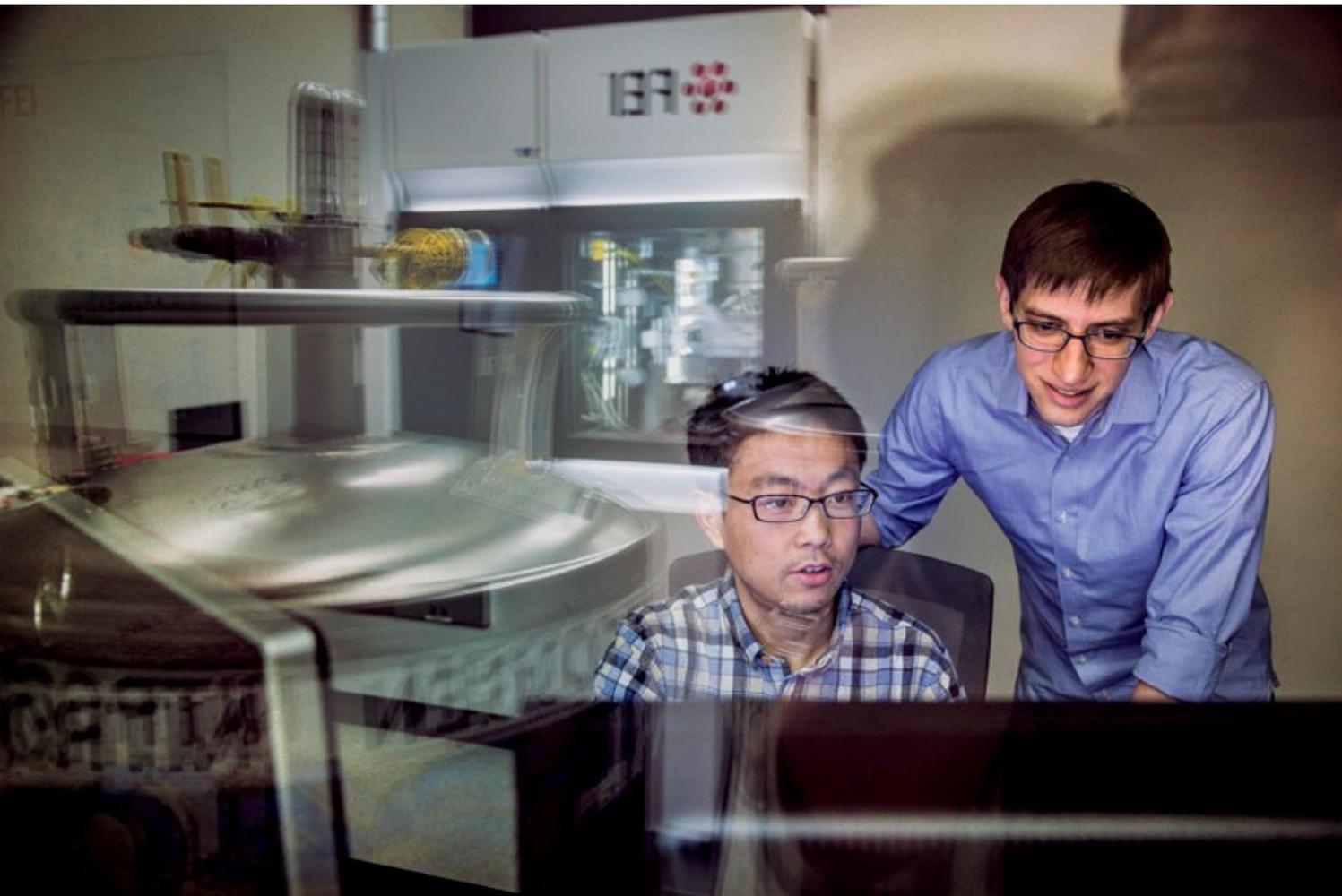
The technique is known as cryo-electron microscopy, or cryo-EM. And it is enabling researchers in labs across campus to investigate biological molecules, and answer scientific questions, that had previously been off limits.

“A lot of impossible projects have become feasible,” says Jue Chen, the *William E. Ford Professor*. She speaks from experience: her own recent cryo-EM investigations are galvanizing research into cystic fibrosis, a debilitating lung disease for which there is as yet no cure.

WHEN IT COMES TO understanding the basic mechanics of life, structure is everything: the shape of a biological molecule dictates what the molecule does and how it does it. Until recently, the gold standard for determining the physical structure of proteins—the molecular workhorses of the body, responsible for performing a nearly endless variety of chores at the cellular level—was x-ray crystallography, a technique that involves turning proteins into crystals and probing them with electromagnetic radiation.

It’s a powerful method, capable of resolving structures at the atomic level. But it is not without its drawbacks.

For one thing, most proteins move through different shapes, or conformations,



as they go about their work. But only one conformation can be crystallized at a time, preventing researchers from analyzing the full range of shapes that allow proteins to do the jobs that nature has assigned them.

Worse, not all proteins are amenable to crystallization: some are too fragile to withstand the process, while others can't be packed into orderly three-dimensional crystals. Yet x-ray crystallography is an all-or-nothing proposition: "You get great crystals, you get a structure. You don't get crystals, you get nothing," says Thomas Walz, who heads the Laboratory of Molecular Electron Microscopy.

Consequently, researchers were left with a vast collection of proteins whose mysteries seemed destined to remain impenetrable, including the so-called membrane

Gregory M. Alushin (right) and postdoc Rui Gong in the cryo-EM control room.

proteins that, among other things, help ferry vital substances in and out of cells.

Cryo-EM, however, changed all that.

Prior to being frozen, proteins destined for the electron microscope are comfortably suspended in a liquid solution that resembles their native environment inside cells, helping to preserve their integrity. Since many different conformations of the same protein can be trapped in ice simultaneously, scientists can see all of them at once.

"It gives me a view of the dynamics of the protein or protein complex I'm interested in," explains Walz—who, when he isn't lending his expertise in cryo-EM to colleagues in other labs, employs the method himself to explore the interactions between membrane proteins, other biological molecules, and the cell membranes they inhabit.



Rockefeller's two cryo-EM units are housed in the Evelyn Gruss Lipper Cryo-Electron Microscopy Research Center. A third unit is on the way.



Jue Chen

New structural data has galvanized research into cystic fibrosis.

Despite these advantages, cryo-EM was not an overnight success. Originally developed in the 1980s, the technique initially produced such fuzzy, low-res images that it was jokingly referred to as “blobology.” But in recent years, improvements to the electron detectors and image processing software that make cryo-EM possible have vastly improved the resolution it can achieve.

As a result, the technology has enjoyed soaring popularity and newfound acclaim—three of the scientists who developed cryo-EM for biology were awarded the 2017 Nobel Prize in Chemistry. And other researchers who once relied primarily on x-ray crystallography—including Roderick MacKinnon, head of the Laboratory of Molecular Neurobiology and Biophysics, who used it in the work that won him his own Nobel Prize, in 2003—have now shifted almost entirely to cryo-EM in hopes of cracking previously insoluble problems.

ONE SUCH PROBLEM involved a membrane protein called cystic fibrosis transmembrane conductance regulator, or CFTR, whose structure Chen spent nearly a decade trying to resolve using x-ray crystallography. After failure upon failure, she finally succeeded just last year, thanks to cryo-EM.

CFTR allows chloride ions to travel across cell membranes, and researchers have long known that mutations in the gene that produces the protein lead to cystic fibrosis, a chronic disease. (Defects in CFTR cause mucus to accumulate in the lungs, with potentially fatal consequences.) They did not, however, know what kinds of changes those mutations caused in the physical structure of CFTR, or how those changes prevented the protein from operating properly—information that could potentially unlock new treatment strategies.

Chen, who heads the Laboratory of Membrane Biology and Biophysics, began working on CFTR in 2008, but try as she might, she could not persuade the protein to crystallize—in part because a section of it, known as the R domain, doesn't have a fixed structure. It flops about from one position to another.

A few years ago, Chen began learning the ins and outs of cryo-EM with help from old hands like Walz.

She initially used the technique to study a form of CFTR found in zebrafish that is virtually identical to the human version; it was easier to work with, and allowed her to refine her methods. Thanks to those preliminary studies, she was ultimately able to get a good look at the human version of CFTR, even determining the location (or locations) of its elusive R domain. Her results, which were published in the journal *Cell* this past March, help explain how a particular mutation interferes with the protein's ability to function, information that could open the door to new therapies.

Most treatments today deal with the symptoms of the disease (mucus buildup, lung infections) rather than its underlying causes. The only FDA-approved drug that addresses a problem with CFTR itself helps less than four percent of cystic fibrosis sufferers—those with a specific genetic mutation. The rest are currently out of luck.

Chen says that further cryo-EM studies could reveal more about the structure of CFTR, and how different mutations cause the protein to fail in different ways. This, in turn, would make it possible to design drugs that bring relief to far larger numbers of patients, something that would have been inconceivable not long ago.

GREGORY M. ALUSHIN, who joined Rockefeller last year as head of the Laboratory of Structural Biophysics and Mechanobiology, is also using cryo-EM to pursue projects that were formerly out of bounds.

Alushin investigates how cells sense mechanical forces: the constant pushing and pulling generated by their own movements and by the environment that surrounds them. These forces can lead a cell to change its motion—encouraging a healthy one to migrate from one position to another, for example, or provoking a cancerous cell to metastasize—and may even cause it to turn genes on or off.

Research has shown that long strings of protein called actin filaments play a crucial role in all of this. The proteins in the filaments, which are strung together in repeating patterns that resemble double-stranded spiral staircases, change shape in response to the forces

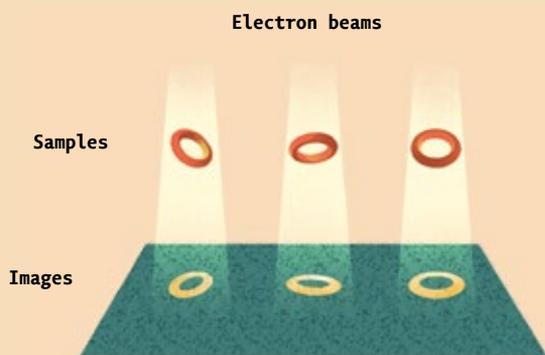
Cryo-EM: How it works

Cryo-EM comes in two flavors: single-particle cryo-electron microscopy and cryo-electron tomography.

1

Single-particle cryo-electron microscopy

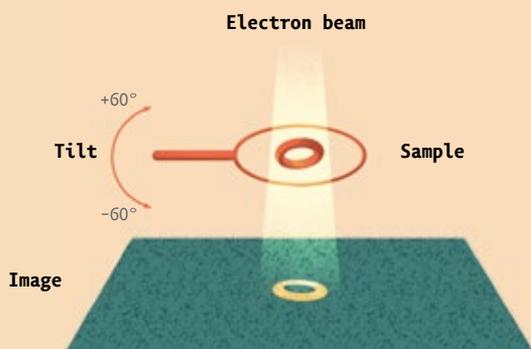
A high-energy electron beam passes through multiple samples containing millions of proteins that have been frozen in a variety of orientations. The system captures a two-dimensional image of each protein, and computer software combines these images to reconstruct a 3D protein structure.



2

Cryo-electron tomography

A single frozen sample is tilted at various angles relative to the electron beam. Rather than capturing single images of millions of proteins, multiple images are taken of far fewer proteins, allowing the software to produce a 3D structure for each of them.



Thomas Walz

Instead of being coaxed to form crystals, molecules can now be imaged in what resembles their native shapes.

they experience. Those changes in conformation invite other molecules known as signaling proteins to bind with them, triggering a series of biochemical events that ultimately lead to changes in a cell's behavior.

Understanding precisely how the proteins in the filaments change shape, and which signaling proteins bind to them, could allow researchers to design drugs that would stop the process in its tracks, preventing tumors from metastasizing, or warding off unwanted changes in gene expression.

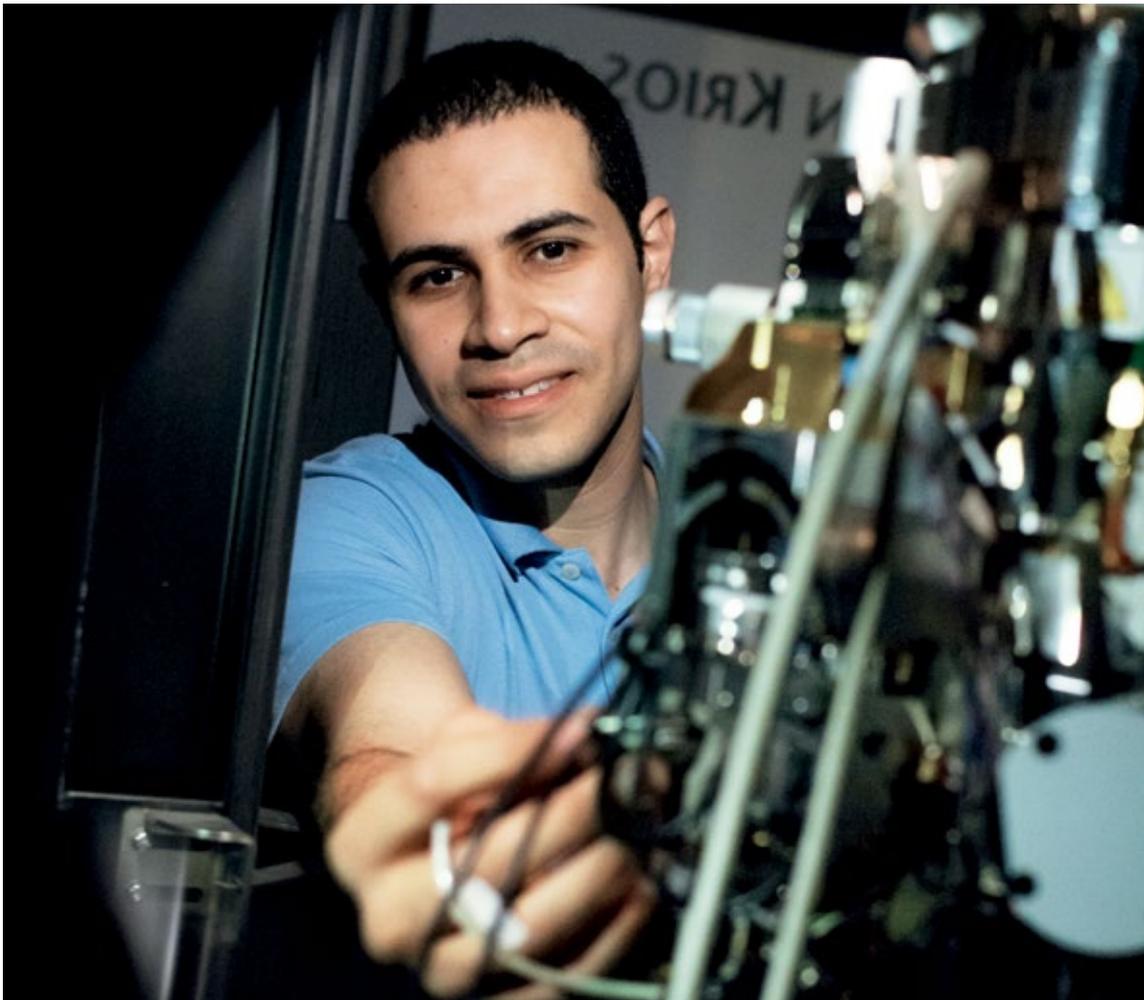
To achieve that level of understanding, however, Alushin first needs to determine the atomic-scale structure of the proteins. Until cryo-EM, that didn't seem possible.

"You can't use crystallography on them, because they don't pack into crystals," he says. And even if they did, he adds, the very act of locking the filament proteins into well-ordered crystals would make it impossible to observe the range of conformations that allow them to do their work.

Freezing the samples solved these problems, and by using specialized "motor proteins" to pull on opposite ends of the filaments as they freeze, Alushin's team can even see how they deform in response to mechanical force.

Much to his surprise, Alushin found that rather than straightening out when stretched, the twisting, double-stranded protein structures comprising the filaments instead developed what looked like "squiggly" regions. He suspects that these deformations are caused by one strand breaking and wrapping around the other, and that the resulting change in shape serves as a target for signaling proteins. He is already conducting further cryo-EM trials to test this hypothesis.

"We know these signals are happening, but we don't know which molecules are involved, or how they interact," he says. "So we're putting together the molecular cast of characters, and observing the conformational acrobatics they undergo."



Mark Ebrahim tending to the equipment.

“We’re putting together the molecular cast of characters and observing the acrobatics they undergo.”

OF COURSE, like any scientific technique, cryo-EM has its own limitations and challenges.

It doesn’t work particularly well on the smallest molecules. The image processing software it relies upon cannot always make sense of all the different conformations in a sample. And it generates so much data—multiple terabytes per project, Ebrahim estimates—that simply storing it all poses a serious challenge. Rockefeller has recently upgraded its high-performance computing capabilities to handle the needs of cryo-EM, and is beefing up its data storage capacity, as well (see “Processing power,” page 5).

Meanwhile, the technology continues to improve, promising even more impressive results to come. And Walz, who has been involved with cryo-EM almost from the start, continues to be amazed by the progress that has already been made since the bad old days of blobology.

“Every day I’m astonished by what can be done,” he says, “and by how easy it has become.” ©

TERRESTRIAL

A photograph of three people sitting on a large, dark rock in a park. The man on the left is wearing a blue shirt and glasses, holding a tablet. The man in the middle is wearing a light grey shirt and glasses, leaning forward. The woman on the right is wearing a white t-shirt and dark pants, looking down at something in her hands. In the background, there are several tall city buildings under a clear blue sky. The word "TERRESTRIAL" is written in large, bold, red letters across the top of the image.



TREASURES

Topsoil is teeming with secret bacterial recipes, some of which could become lifesaving drugs. Chemists are using new tools to dig deep into this hotbed of medical potential.

By Wynne Parry

S EAN BRADY'S STAINLESS-STEEL incubator contains no sand, no moldering leaves, no silently squirming worms. But open its glass door and inhale: there's that unmistakable, warm scent of moist earth.

Stacked in neat towers on the shelves inside are plates fuzzy with colonies from which the earthy perfume emanates. These are not just any bacteria; they are—potentially—the key to an entirely new generation of medicines. Master chemists, they serve as manufacturing plants for an astonishing range of chemical substances normally produced by soil microbes. For Brady, a chemist himself, these dirt-derived compounds are full of potential, capable of yielding killer antibiotics, powerful cancer chemotherapies, or blockbuster anti-depressants.

Soil is a logical place to look for obscure molecules: a single gram, less than a teaspoon, may contain thousands of species of soil bacteria—an immense source of untapped medicinal chemistry. “It’s the largest, most biodiverse reservoir of bacteria on the planet,” says Brady, “and it’s very easy to get.”

Dirt microbes are not easy to work with in the lab, however, and much of their pharmaceutical power has long been beyond human reach. But Brady, whose team has spent ten years developing new methods to mine soil for new molecules, is at the forefront of a drug renaissance.

His approach, a calculated combination of microbiology, chemistry, and computational biology, has opened the door to a world of chemistry no scientist has seen before.

B RADY IS NOT alone in his quest for esoteric soil substances. In fact, the ground beneath our feet is an ancient pharmacy that humans have exploited since long before we peered through our first microscope. Archaeologists, for instance, have found traces of tetracycline, an antibiotic produced by soil bacteria, in 1,500-year-old human bones from Sudan, suggesting that our North African ancestors were brewing and ingesting the drug on a regular basis.

The modern effort to produce this and similar compounds kicked off after 1928, when the doctor and

scientist Alexander Fleming identified penicillin from airborne fungi. Following his discovery, other scientists began extracting lifesaving medicines from microorganisms, mainly soil bacteria. Among them were actinomycin D, a chemotherapy ingredient deployed against several types of cancer; streptomycin, an antibiotic that became the first treatment for tuberculosis; and other antibacterial drugs used to treat infections from acne to malaria.

Mid-century scientists were at the epicenter of an innovation boom that lasted a couple of decades—until the field ran up against an insurmountable obstacle. To be able to peruse a bacterium’s molecular contents, scientists first had to grow it in the lab, on plates or in shaker flasks; but most soil microbes won’t adapt to life inside an incubator. “The problem with growing unusual bacteria is that we don’t know what most of them eat, if they need companions of another species, or more space,” Brady explains.

So, from Fleming’s time until relatively recently, microbiologists had to concentrate their efforts on what turned out to be a relatively small number of domesticated species. To date, only a fraction of the world’s soil bacteria have been cultured—the rest are a black box.

Brady, who is the Evin Associate Professor, has found a way to peer inside that box, however. Rather than trying to cultivate obscure and delicate microbes, his lab focuses on the soil itself, searching it for microbial

Sean Brady



It's now possible to extract a bacterium's recipes for making chemicals without the messy, failure-prone process of actually keeping that bacterium alive.

Zachary Charlop-Powers prepares dirt samples for sequencing.



DNA that contains the instructions for making new molecules. Thanks to new technology, it's now possible to extract a bacterium's recipes for making chemicals without the messy, failure-prone process of actually keeping that bacterium alive.

Among Brady's peers, many have been successful in trawling bacterial genomes for drug-like substances using cutting-edge genome sequencing approaches. But Brady has set himself apart by looking not at the bacteria themselves, but rather at the DNA of organisms never grown in a lab.

"All Sean needs is DNA, and that's the beauty of his approach," says Jon Clardy, a professor at Harvard Medical School and the mentor who inspired Brady's interest in the chemical-engineering potency of bacteria. "The strategy is conceptually very simple, but extraordinarily sophisticated from a technological standpoint."

THINK OF IT this way: if forensic scientists struggle to decode human DNA fragments hidden within, say, a piece of torn clothing from a crime scene, microbiologists like Brady face an even greater challenge examining DNA from dirt, which contains genetic material from thousands of unknown bacterial species—not to mention other subterranean beings like fungi, single-celled protozoa, microscopic worms, scraps of plants, and the occasional insect. To make progress, Brady has had to create new methods to find the DNA of interest,

as well as brand new computational technologies to analyze it. Among other things, his lab uses highly specialized software to scan DNA sequence data derived from thousands of different soil environments for genes likely to produce agents with desirable qualities—molecules that mimic certain other molecules, for instance.

It's a highly sophisticated approach that requires a litany of expensive equipment, technical know-how, and, to get things started, a shovel (see "How to mine dirt for drugs," page 34).

"Sample collection is easy," says Zachary Charlop-Powers, a computational scientist and former post-doc in Brady's lab. "The vast majority of microbes are found in the top 12 inches of soil, so we literally just dig." He and his coworkers have collected dirt from the Rockefeller campus, from parks throughout the city, and from distant sites where traveling lab members have found themselves.

The work gets more complicated once the dirt arrives in the lab. Charlop-Powers and his coworkers extract bacterial DNA from it, and from there, the search may go in one of two directions. The scientists may conduct a targeted search for pharmaceutical molecules, guided by familiar chemistry (in one project, for example, they identified an agent similar to anti-cancer medicines called anthracyclines that they hope will be potent against tumors that no longer respond to those drugs). Or, in some cases, they take the "surprise me"

approach, randomly testing all the molecules a bacterium can make for pharmaceutical activity.

Once they have identified promising pieces of bacterial DNA, the scientists move on to manufacture the corresponding molecules, a job that is in fact outsourced to a cooperative species of soil bacteria like *Streptomyces*, microbiology's answer to the lab rat.

Reaching into the Brady lab's fragrant incubator, Ian Woodworth, a research assistant, grabs a plate covered with orange-gold colonies of *Streptomyces*. Mixed in with their normal DNA, these bacteria harbor genes from other microbial species—molecular recipes that Brady's team has found in a search for new drugs against tuberculosis. In particular, the researchers hope that some of these genes, now activated by the *Streptomyces*, will yield molecules capable of killing potentially deadly strains of *Mycobacterium tuberculosis*, the germ that causes lung infections.

Woodworth carefully picks *Streptomyces* colonies off the plate and releases them into broth-filled flasks, where the lab-friendly germs will be fed, aerated, and shaken for days. These colonies are essentially small chemical plants, pumping out their assigned

molecules. (Of their own volition, *Streptomyces* also make geosmin, an oily molecule that gives these bacteria their earthy smell). From the enriched broth, Woodworth and his colleagues will later produce concentrated extracts of the compounds, which they will feed to tuberculosis bacteria. With any luck, the bacteria will die.

The same approach has led Brady and his coworkers to a number of molecules that they're now developing as experimental drugs and testing in animals. Among them is what may be an entirely new class of antibiotics, one that appears frequently in soil samples collected across the country. These drugs, the scientists have found, have the potential to kill drug-resistant strains of *Staphylococcus aureus*, a germ that often causes dangerous infections in hospital patients.

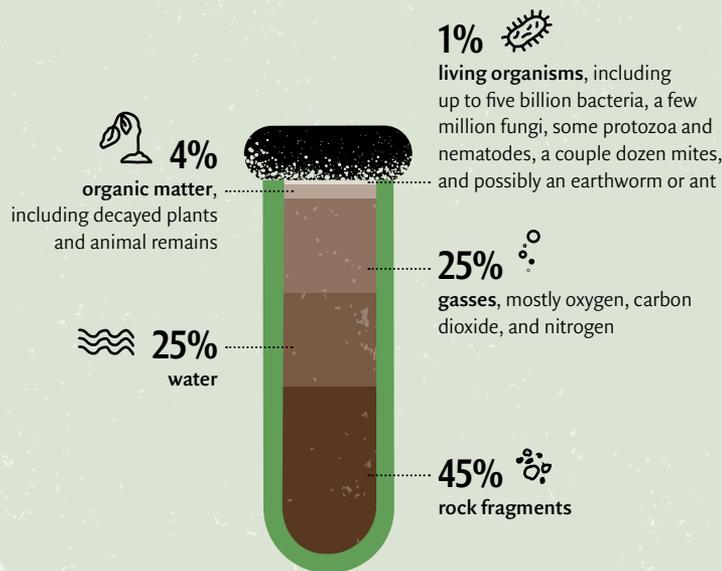
"Sean has really been at the forefront of pulling relevant sequences out of the mishmash of DNA within soil," says Jason Crawford, an assistant professor at Yale University whose lab, like Brady's, uses genomics-based methods to discover new microbial compounds. "This has allowed him to do some wonderful things identifying and activating these natural products."

3,000 lbs
 Mass of bacteria found in one acre of rich soil

99.999%
 Proportion of bacterial species on Earth that have yet to be identified

What's in dirt?

Although the exact composition of soil varies by geography, climate, season, and many other factors, a typical handful of your backyard might contain:





How to mine dirt for drugs

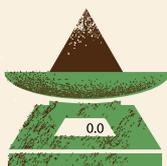
To study molecules produced by rare soil bacteria without growing these bacteria in the lab, scientists obtain their DNA directly from dirt.

1

Topsoil samples are collected...



...and processed in the lab to extract bacterial DNA.



2

The DNA is sequenced and computationally searched for promising genes.



A gene of interest is engineered into easily-bred *Streptomyces* bacteria...



3

... that are grown in bulk to manufacture the molecule encoded by the gene.



The molecule is isolated from the broth, purified, and tested for medical activity.



IN PRINCIPLE, BRADY'S approach could tip him off about new ways to treat nearly any type of disease. But there are good reasons why his lab pays particular attention to antibiotic drug candidates—molecules that look like they might have what it takes to kill germs in ingenious ways.

Despite having well over 100 antibiotics at their disposal, infectious disease doctors need more. Bacteria multiply quickly and are always mutating, which means the forces of evolution are keeping modern medicine on its toes: each time a new antibiotic is introduced, the microbes it targets begin devising ways to thwart it.

Health experts warn that humans are in danger of falling behind, and we may soon live in a world where antibiotics are of little use, and people die from common infections or minor injuries. Unsettlingly, a 2014 report by the World Health Organization states that “a post-antibiotic era... far from being an apocalyptic fantasy, is instead a very real possibility for the 21st century.”

The answer to this threat could be new antibiotics capable of conquering drug-resistant bacteria. Such pharmaceuticals most likely exist in nature, Brady says, because microbes competing for resources are in a constant tug-of-war. “The same drug-resistance mechanisms we see in hospitals are being used by soil bacteria trying to dodge their natural enemies,” he says, “and those enemies will in turn come up with new antibacterial substances.”



Streptomyces bacteria, fresh from the incubator; Sean Brady and research assistant Melinda Ternei sorting through cultures.



SINCE DARWIN'S TIME, ecologists have ventured across continents to explore new realms of biological diversity. Generations of microbiologists have trod this path as well.

After Fleming discovered the first antibiotic without leaving his lab in a London hospital, for example, other medical agents emerged from remote sites. The antibiotic erythromycin was obtained from bacteria residing in soil from the Philippines. Rapamycin, an immunosuppressant used to prevent the rejection of organ transplants, originated from the earth of Rapa Nui, more commonly known as Easter Island. And epothilone, a widely used anticancer agent, arose on the banks of South Africa's Zambesi River.

"The field was based on the idea you would put on your explorer outfit and go hike the world looking for samples," says Brady, whose lab has faithfully scrutinized dirt samples procured by volunteers from the deserts of Arizona, the Brazilian rainforest, and beyond.

Lately, however, he's been sticking closer to home.

With help from the nonprofit Natural Areas Conservancy, Brady's team has collected close to 300 soil samples from a variety of ecosystems within New York City parks, none more than 30 miles from the lab.

And, somewhat surprisingly, it turns out that they've got plenty to work with right here: New York's topsoil is as cosmopolitan as the city that rests upon it. Among the DNA segments the researchers have extricated from park dirt, many look eerily familiar. Brady and his lab

members have seen genes similar to those encoding erythromycin, rapamycin, and epothilone among other existing medicines, all of which were originally derived from microbes found elsewhere on the globe.

This suggests, according to the researchers, that the microbes from which many of our workhorse drugs were originally derived are not unique to their native environments. For example, *Sorangium cellulosum*, the African-born microbial architect of epothilone, may well have cousins and uncles in New York.

It's excellent news for microbiologists with dwindling travel budgets or concerns about disturbing the world's vanishing ecosystems.

"The fact that we can turn up the same potentially useful molecules in our own backyards suggests it may be time to shift away from a voyager approach," Brady says, "and focus instead on the incredible abundance of unfamiliar genes that can be found right here."

And indeed, despite the geographic commonalities, there's a tremendous amount of unexplored chemistry among Brady's samples. Of the millions of sequences the scientists have produced from New York City park dirt, less than one percent have matched with known genes in public databases. Lurking among the rest could be the next revolutionary pharmaceutical.

"There's a tremendous amount we don't recognize," says Charlop-Powers. "A lot of new and potentially very useful chemistry is still out there." ☉

B E Y O
N D R
E C O G
N I T I
O N

BY ALEXANDER GELFAND

Those suffering from prosopagnosia live in a bewildering world, inhabited by people whose faces are impossible to tell apart. Profoundly mysterious and virtually untreatable, the disease is leading neuroscience into uncharted nooks of the brain.

HEATHER SELLERS IS A TERRIFIC CONVERSATIONALIST.

She's attentive, she's engaging, and she makes constant eye contact. Within moments of meeting her, you'll feel as if you've known her for years.

Just don't be surprised if, the next time you see her, she has no idea who you are.

That was the case when Sellers came to The Rockefeller University in March to meet with Winrich Freiwald, head of the Laboratory of Neural Systems; and Christina Pressl, a physician who does clinical research in Freiwald's lab.

Sellers, a professor of English at the University of Southern Florida, had last seen Freiwald and Pressl just a few months earlier while speaking at a Rockefeller event. But while she recognized Freiwald on sight, she drew a complete blank on Pressl. "You have to tell me who you are," Sellers said, as Pressl wrapped her in a hug.

Sellers' vision is fine, and she is hardly an amnesiac. As a writer—she has published several books, including the memoir *You Don't Look Like Anyone I Know*—she





Winrich
Freiwald

Face recognition is an ideal model for understanding the brain's social, emotional, mnemonic, and cognitive functions.

demonstrates tremendous recall, painting scenes from her childhood replete with physical detail.

She is, however, severely face blind.

The technical term for her condition is prosopagnosia, from the Greek for “face” (*prosop*) and “lack of knowledge” (*agnosia*); and it is a disorder that Freiwald and Pressl are investigating on a variety of levels, and for a variety of purposes.

For one thing, figuring out why some people cannot recognize faces could lead to better tools for diagnosing and treating prosopagnosia, and to a more detailed picture of visual processing at large. But it could also result in a deeper understanding of how the brain organizes information and responds to stimuli in general—an understanding that could someday help explain and address numerous disorders of the mind.

NOTWITHSTANDING THE RATHER blunt literal meaning of the term, people who suffer from face blindness can in fact see faces; they just can't recognize them. Sellers, for example, does not see an amorphous blur between your ears while she is talking to you. But if she turns away and closes her eyes, she cannot form a coherent image of your face in her mind's eye. “I can't remember it at all, even for a second,” she says. For Sellers, there is no such thing as a familiar face; not even her own, reflected in a mirror.

This does not mean, however, that Sellers is incapable of recognizing people. Like many developmental prosopagnosics—that is to say, individuals who were born with the condition, as opposed to those who acquired it through damage to the brain's temporal lobe—she has found ways of compensating. For example, she has a knack for identifying people by the way they walk; and the more non-facial clues she has, the better she does.

When Sellers recognized Freiwald in March, for instance, he was in his office—a clear contextual giveaway. He was also, as always, extremely tall. “Outliers are easy,” Sellers says.

Pressl, alas, was in a room with five or six other women; and if picking a single face out of a crowd can be tough when you aren't prosopagnosic, imagine how difficult it must be when you are. To make matters worse—for Sellers, if not for her—Pressl has a highly symmetrical face. And as Sellers explains, their very lack of disproportion makes symmetrical faces even harder to place.

According to Brad Duchaine, a psychologist at Dartmouth who diagnosed Sellers, prosopagnosia comes in a variety of flavors. While all prosopagnosics have difficulty recognizing faces, some also have trouble with related tasks, such as identifying facial expressions. And the condition occurs with different degrees of severity, pointing toward what Freiwald calls a “face-blindness spectrum.”

Prosopagnosia was once thought to be exceedingly rare, and almost always caused by brain damage. Today, however, experts believe that as many as two percent of the population, or six million people in the United States alone, may be face blind, with most cases

being developmental. And the condition has profound consequences both for prosopagnosics themselves and for society at large.

Sellers emphasizes that even a mild case of face blindness can be socially devastating. In her memoir, she describes how her own extreme version made dating impossible, caused her to constantly mistake her ex-husband for a stranger, and led people she had known for years to assume she hated them because she was forever snubbing them. By the time she was diagnosed in her mid-thirties, she was so used to hiding her disability—and so ashamed of it—that it took her more than a year to come out publicly as a prosopagnosic. Having conquered her own fears, she now strives to help others, raising awareness of the condition and sharing coping strategies with fellow sufferers.

Freiwald, meanwhile, notes the implications for the criminal justice system. The London Metropolitan Police famously use so-called super recognizers, who are as adept at recognizing faces as prosopagnosics are not, to identify suspects on surveillance videos.

In this country, on the other hand, eyewitness misidentification accounts for more than 70 percent of the convictions overturned by DNA evidence. It's a statistic that suggests witnesses, not to mention police officers and border control agents, ought to have their face-recognition abilities evaluated as a matter of course.

At the very least, Freiwald says, detectives and prosecutors could attend more carefully to the science of facial recognition when relying on eyewitness identification, a point made in a 2014 report by the National Academy of Sciences to which he contributed.

Meanwhile, that science continues to evolve. Duchaine explains that while researchers have parsed the general features and the overall sequence of events involved in normal face recognition, many of the details remain a mystery. And without a comprehensive and detailed understanding of how face recognition occurs under ordinary circumstances, face blindness remains hard to diagnose and difficult to treat.

Which is partly why the work that Freiwald and Pressl are undertaking is so crucial.

FACE BLINDNESS WAS first identified in the 19th century, but its neurological underpinnings didn't begin to emerge until much later.

In the 1980s, for example, research on monkeys showed that some of the neurons involved in visual processing responded only when the monkeys saw faces, as opposed to other kinds of objects (cars, spoons, oranges). And in the late 1990s, MIT neuroscientist Nancy Kanwisher used functional magnetic resonance imaging (fMRI) to demonstrate that a specific region of the human brain becomes active only in response to faces, as well.

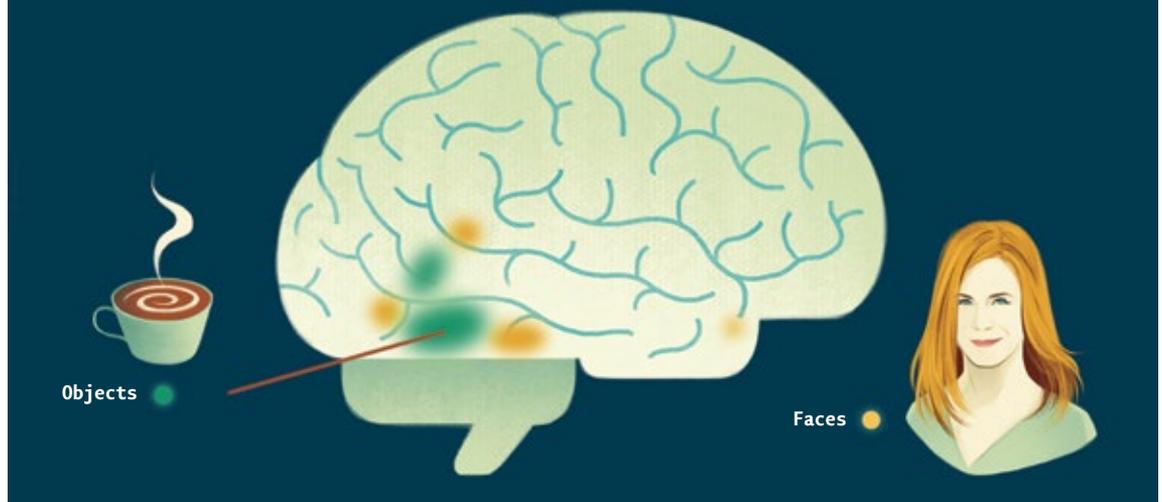
It was Freiwald who successfully merged both lines of investigation a decade later, while working as a postdoc in Kanwisher's lab. He and his collaborator, Doris Tsao, who is now at Caltech, combined fMRI studies with recordings of individual neurons in the brains of macaques. These experiments revealed that a collection of small face-sensitive areas distributed throughout the temporal lobe—areas that he and Tsao dubbed “face patches”—were composed almost entirely of face-selective cells.

Since coming to Rockefeller in 2009, Freiwald has continued to combine imaging studies with recordings of individual cells to explore the brain's face-processing mechanisms.

His team has demonstrated that different face cells and patches perform different face-recognition tasks. Some cells, for example, respond only to faces seen in profile, while others specialize in detecting the differences in contrast that set the eyes apart from the forehead, nose, and cheeks. (Watching footage of a face cell in action is surreal: the cell's electrical activity is represented

Faces are special

The regions in the brain that respond to the sight of a face—called face patches—are separate from those tasked with recognizing other objects. Each face patch contributes to a different aspect of face perception, like responding to particular features or interpreting facial expressions.



sonically by a kind of crackling static; and while it seems incredible that a single neuron could be so discriminating as to squawk only when presented with a face in profile, the video does not lie.)

In addition, Freiwald and his colleagues recently discovered that some face patches respond to facial motion, while others react most strongly when shown faces and bodies together. They have also shown that most face patches communicate with one another, passing information from one part of the face-recognition network to the next like a neural bucket brigade. For familiar faces, the end result carries with it a wallop of information drawn from memory, as our brains conclude that we are looking at not just any visage, but at that of a particular individual with a specific identity (your mom, your dad, your boss). The appropriate reaction can then follow.

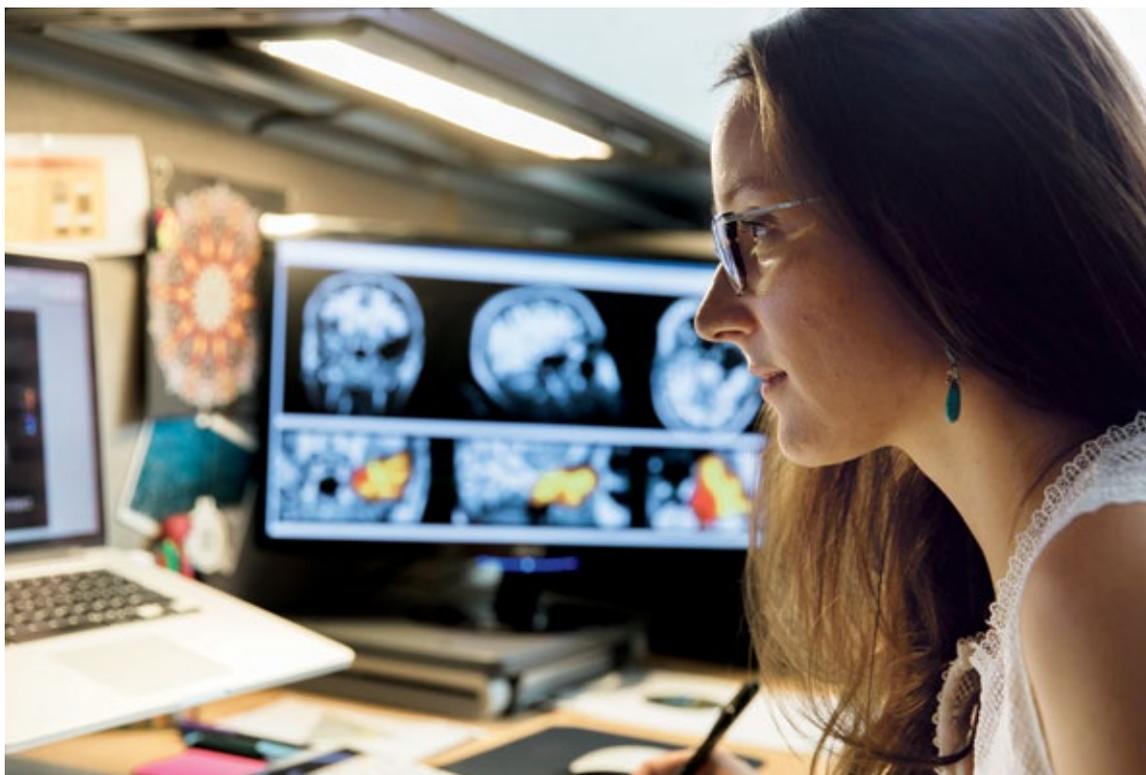
Pressl, who trained as a radiologist, draws on these insights to investigate face recognition in people.

With help from NYU's Comprehensive Epilepsy Center, she is scanning the brains and testing the face recognition skills of individuals with temporal lobe epilepsy. By working with patients before and after they undergo brain surgery, Pressl is teasing out the effects of both the disease and its treatment on facial recognition, thereby gaining a deeper understanding of prosopagnosia as well.

She is also analyzing millions of patient records in hopes of arriving at a more accurate estimate of the number of prosopagnosics

in the United States. And she is developing a shorter and hopefully more effective test for the condition, which can be surprisingly difficult to diagnose—in part because many doctors still aren't familiar with the disorder ("I did not learn about prosopagnosia in medical school," Pressl admits), and in part because there is no gold standard for identifying it. The first neurologist whom Sellers saw, for example, dismissed the idea that she could have such a supposedly rare ailment. And the second gave her a test that someone suffering from acquired prosopagnosia probably would have failed, but which she, thanks to her battery of coping skills, managed to pass.

BY ILLUMINATING THE neural mechanisms underlying face perception, Freiwald and Pressl hope to contribute to the development of new and better treatments. Current therapies for face blindness are limited to cognitive training exercises that require daily practice, offer



Christina Pressl conducts clinical work in the Freiwald lab.

JACOB PRITCHARD

The moment we set eyes on someone, we immediately begin assessing their attentiveness, their mood, even their trustworthiness, and we calibrate our responses accordingly.

only modest benefits, and fade with time—hardly an appealing combination. “It’s like going to the gym, and it doesn’t stick. I’m not doing it,” says Sellers.

Their investigations also promise to shed light on the basic organization of the brain itself, revealing how information moves through neural networks, for example, and how separate systems like visual perception, memory, and attention are integrated.

Yet the implications of their research could extend even further.

Face recognition plays a profound role in social interaction. The moment we set eyes on someone, we immediately begin assessing their attributes—their attentiveness, their mood, even their trustworthiness—based on their facial gestures and expressions, and we calibrate our responses accordingly.

What’s more, the face-processing network and the various neural systems it touches upon, like memory and attention, all operate automatically: we return a smile, follow a person’s gaze, infer their intent, and call up their personal file from our memory banks without any conscious thought. Yet unlike many of those systems, face recognition is localized in specific areas of the brain and is relatively easy to probe, making it an ideal model for understanding social, emotional, mnemonic, and cognitive function.

As a result, a thorough grasp of face recognition—and face blindness—could help scientists understand a whole range of disorders characterized by atypical social or emotional responses, including autism and some forms of mental illness.

“This really is an amazing opportunity to get deep into the brain mechanisms of our social and emotional minds,” Freiwald says.

TO CAPITALIZE ON that opportunity, however, researchers must first figure out precisely how normal face recognition works, and how the process goes awry in prosopagnosia. And that, in turn, will require a solid grasp of the

genetic basis of face blindness—something that scientists currently lack.

According to Duchaine, roughly half of all developmental prosopagnosics report having family members who also have difficulty recognizing faces. Yet at present, researchers have no idea which genes might be responsible for causing the condition.

Moreover, because the facial-recognition network comprises many interconnected areas in the brain, the opportunities for disruption—for abnormalities in one face patch or another, or for faulty connections between patches—are vast. Any number of genes, acting independently or in concert, could potentially lead to the neural impairments that ultimately give rise to prosopagnosia in a particular individual.

To begin to explore the genetics, Freiwald, Pressl, and Duchaine are therefore collaborating on a study in which developmental prosopagnosics will have their genomes sequenced and their face-recognition abilities tested. The resulting data will ultimately be compared with that of a larger pool of unaffected people. This should allow the team to ferret out the specific genetic variants associated with face blindness.

That kind of information could eventually allow scientists to tell someone like Sellers precisely which genes and neural processes are responsible for her condition. And it would bring researchers that much closer to developing better tests and therapies for face blindness.

“That,” Freiwald says, “would be an amazing level of understanding.”



About the artist

Illustrator **Yuko Shimizu** on seeing and drawing faces

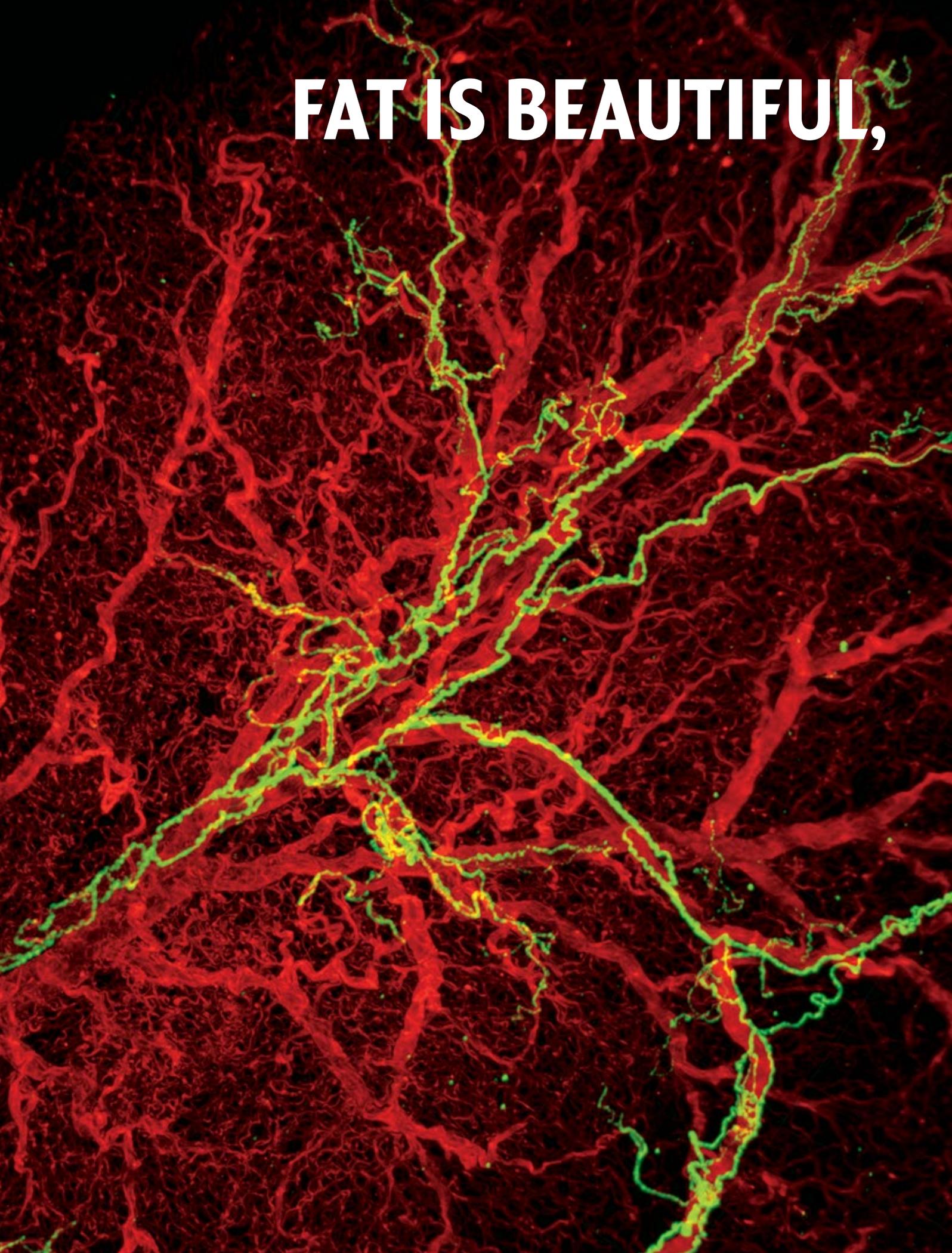
Although Shimizu was born with prosopagnosia, it wasn’t until way into adulthood that she discovered there was a name for her condition.

Growing up in Japan, she played Fuku Warai (“Lucky Laugh”) with her family around each New Year: akin to pin-the-tail-on-the-donkey, blindfolded players try to place noses, eyes, and other features on an outline of a face, and comedy ensues. That’s still what she thinks of when she tries to explain to people why she probably won’t recognize them later: “I can see your face clearly,” she says, “but its overall image won’t stick.” To remember it, she memorizes each feature individually, then pieces together the clues.

From her New York City studio, Shimizu has made art for everything from Pepsi cans and Gap t-shirts to articles in *The New York Times*, *Rolling Stone*, and *The New Yorker*. She often does portraits, but never from memory.

“To draw Brad Pitt, I would need tons of photos taken at different angles,” she says. “To me, it would be the same as drawing an apple—a face is just another object drawn from observation.”

FAT IS BEAUTIFUL,



ESPECIALLY BEIGE FAT

By David Noonan

Inside the energy-burning cells that just might hold the answer to some of our biggest health problems.

AS RECENTLY AS NINE years ago, fat was fat. It stored energy, caused disease, and made people feel bad about themselves. Drooping over the belts of millions of Americans, raising our blood pressure and decreasing our insulin sensitivity, fat was simply a burden, something to lose.

It turns out, however, that the decidedly unglamorous word “fat” belies a biological system as elegant, important, and challenging to understand as any in nature. Counterintuitively, fat, the reviled driver of the obesity epidemic, could potentially be harnessed in new treatments for a variety of obesity-related conditions, including type 2 diabetes, cardiovascular disease, and certain cancers.

It’s a remarkable possibility that stems from the fact that not all fat is the same. Scientists have recently discovered that there are not one, not two, but three kinds of fat: white, brown, and beige. And while white fat has long been known to cause all sorts of trouble, brown and beige fat may actually promote good health.

In addition to just storing energy, brown and beige fat cells also consume it to produce body heat. For Paul Cohen, who heads the Laboratory of Molecular Metabolism, this makes fat itself a potential solution to an ongoing, ever-worsening health disaster.

Interior architecture of a fat blob criss-crossed by blood vessels (red) and nerve cells (green).

WHEN IT COMES TO public health, few problems are bigger than obesity. According to the most recent data from the World Health Organization, more than 600 million adults worldwide are obese and another 1.3 billion are overweight. Findings from 195 countries, published in the *New England Journal of Medicine* in June, pegged the global prevalence of obesity at 12 percent for adults and five percent for children, more than twice the rates of 1980.

This crisis, which inspires Cohen's research, is rooted in a deceptively simple equation. "If your energy intake chronically exceeds your energy expenditure," he says, "that extra energy has to be stored somewhere. And it tends to be stored in fat."

Many researchers have focused on the food intake part of that equation, which has led to the development of several so-called diet drugs that act on the brain to curtail our desire to eat. But these therapies have been dogged by complications: "If you want to regulate appetite," says Cohen, who is the *Albert Resnick, M.D., Assistant Professor*, "not only is it hard to get dramatic weight loss, it's hard to do so without side effects, because the brain is so complex."

He chooses instead to concentrate on energy expenditure. Cohen's ultimate goal is to develop therapeutic methods for manipulating brown and especially beige fat to burn more energy, in order to protect people against obesity and its array of accompanying metabolic diseases.

BEFORE 2009, energy-burning brown fat was thought to exist only in certain small animals and in newborn humans, who need it to maintain their body temperature without shivering (brown and beige fat are both activated by exposure to cold). Its discovery that year in adults made headlines and launched a new era in the study of fat, which is technically known as adipose tissue. Then three years later, the discovery of beige fat in humans super-charged the growing field. Beige fat, found mixed in with brown fat in the neck and along the upper spine, has greater potential than brown fat, Cohen says, because it's highly inducible—it changes quickly from a totally dormant state to a very active, energy-burning (or thermogenic) state. "From a therapeutic standpoint that's exciting," says Cohen, "because you could potentially toggle that switch back and forth."

He began his obesity research working on both the intake and expenditure sides of the energy equation. He pursued his Ph.D. in the lab of Rockefeller's Jeffrey M. Friedman, who in the 1990s discovered the appetite-controlling hormone leptin, earned his medical degree from Weill Cornell Medicine (he's a cardiologist), and did his postdoc with Bruce Spiegelman at Harvard Medical School. He's on his own now, leading a team of 12 grad students and postdocs, trying to unlock the inner workings of beige and brown fat, seeking to identify and understand the genes and proteins that govern their functions.

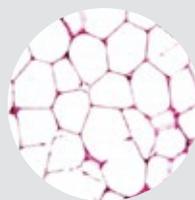
Cohen is particularly interested in a protein called PRDM16, which is known to be important both for the development of beige fat cells and for their ability to burn energy. Mice who lack the protein are unable to activate their beige fat and end up with many of the same features seen in human metabolic disorders, including insulin resistance, weight gain, inflammation, and, as suggested by Cohen's recent and yet unpublished experiments, hypertension.

While many scientists in the field are trying to understand how PRDM16 functions, to Cohen the protein is more of a tool. For his experiments, he breeds mice that either lack PRDM16 or produce it at natural or elevated levels. He and his team then use these different animals to study how beige, brown, and even white fat cells behave under various conditions.

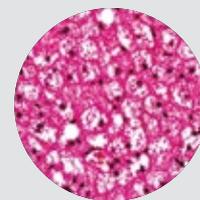
Their goal is to develop interventions that augment beige fat activity by manipulating PRDM16. Accomplishing this without producing significant side effects might be easier said than done, however, because PRDM16 is expressed in a wide variety of cell types, and plays an important role in crucial biological processes, including the differentiation of stem cells into mature cells. "For example," says Cohen, "PRDM16 is involved in the development of cells in the blood lineage, so there is the very real concern that if you activated it in those cell types you could get leukemia."

The fat spectrum

Adipose tissue comes in three shades with different properties:



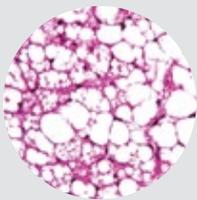
WHITE FAT is the most abundant, accounting for up to one-fourth of a normal-weight person's body mass. Mainly found under the skin and within belly deposits, its main function is to store energy.



BROWN FAT is scarcer and wanes with age. Stored in small deposits throughout the body, it converts energy stored as fat into heat, keeping us warm when temperatures drop.

What if dropping the wrong kind of pounds will make us less metabolically fit?

JACOB PRITCHARD



BEIGE FAT is also energy-consuming, and can be activated either by cold or by certain hormones. Discovered in humans only five years ago, it is mixed in with brown fat in the neck and along the upper spine, and embedded within white fat.



Paul Cohen trained as a cardiologist and continues to see patients while running a lab.

A less risky proposition would be to figure out a way to make a drug that would target the protein only in fat cells. It's an approach that could ultimately lead to the holy grail of fat research—new drugs to treat obesity and its related diseases.

"It's conceivable," Cohen says, "that if we identified a kinase that regulated PRDM16 in fat cells and found a way to modulate it, we might ramp up the metabolic activity of beige fat."

THE BIOLOGY OF FAT is intricate in itself, yet Cohen says that to fully understand how adipose tissue functions, one needs to consider its interactions with the nervous system, which plays an important role in letting fat cells know how much energy to store and how much to expend. Jingyi Chi, a grad student in the lab, set out to examine the tissue-level organization of the fat, including its innervating nerve cells. She hit a roadblock almost immediately. Scientists who study fat were missing quality imaging—few people had developed tools to look at adipose tissue in detail. So she borrowed and modified a revolutionary three-dimensional

tissue imaging system known as iDISCO, developed in another Rockefeller lab to study the brain, that makes it possible to “clear” the tissue of lipids and reveal the structures underneath.

“When people think about adipose tissue, it’s just a lump of fat,” Chi says. But her experiments exposed a lot more: a stunning biological architecture within the lump, including long, luminous green neurons and a glowing red web of blood vessels. And beyond being an aesthetic revelation, the results were scientifically stunning.

For instance, the images revealed that nerve cells form projections called neurites—which Chi says are necessary for the activation of the beige fat—to a different extent depending on the presence or absence of PRDM16 in the fat cell. “This suggests some dialogue where, if you have PRDM16 in a fat cell, it somehow guides the neurites to the site,” Cohen adds. “And if you take it away you don’t have those neurites.” His team is now working to identify the molecular components of this dialogue, insights into which could offer yet another way to manipulate beige fat and fight obesity.

LIKE EVERY GOOD SCIENTIST, Cohen is careful not to oversell his research, but his enthusiasm for the therapeutic potential of beige fat is unmistakable. He is especially interested in it as a possible weapon against diabetes. More than 29 million people in the U.S., nine percent of the population, have the disease, one of the grim hallmarks of obesity. One of Cohen’s ideas for a possible treatment grew out of the observation that mice that lack PRDM16 become insulin resistant at the level of the liver, a condition that can lead to type 2 diabetes. This was a strong and consistent finding, and Cohen and his lab members wondered if there might be some kind of molecular signal from beige fat to the liver that regulates insulin sensitivity—a signal that is disrupted in the absence of beige fat activity.

Sean O’Connor, another grad student in the lab, confirmed their suspicion by growing liver cells in a petri dish and bathing them in media recycled from other dishes used to grow fat cells. The results from his deceptively simple experiment showed that molecules secreted by white fat cells increased glucose production and decreased insulin sensitivity, which is bad, while those produced by beige fat cells had the opposite effect, improving insulin sensitivity and reducing glucose levels, which is good.

The findings are preliminary, Cohen notes, but “this suggests that there is some direct interaction between the fat and the liver that’s important. What we hope is that this will lead to the discovery of a new beige fat-derived factor that regulates hepatic insulin sensitivity.”



Jingyi Chi preparing mouse fat for imaging (above). Cohen (right) with research assistant Audrey Crane.



ENCOURAGED BY THESE FINDINGS, Cohen is turning his attention to other obesity-related diseases—for example, he is exploring the role of beige fat in regulating blood pressure and slowing the growth of breast tumors. But perhaps the most surprising takeaway from his work is that, when it comes to health, how much you weigh is less important than the type of fat you have. Even where you carry that fat matters: visceral fat, found in the belly, is associated with metabolic disease, while subcutaneous fat, on the hips and elsewhere, likely contains some beige fat and may be considered healthy.

As a result, some people who are far heavier than their recommended weight, including a former female patient of Cohen's who weighs well over 300 pounds, don't have diabetes, high blood pressure, or any of the other disorders that plague most obese people. They are what he calls MHOs, the metabolically healthy obese, and their extra pounds tend to be distributed around their hips rather than in their bellies. Meanwhile, other people who are at or below what's considered a healthy weight nevertheless suffer from obesity-related diseases. India, for example, is experiencing a huge

increase in the incidence of type 2 diabetes, Cohen says, and there the typical patient "might weigh less than 150 pounds, but have a slight paunch." That paunch, made up of visceral fat, appears to be enough to cause serious problems.

All of which turns our national obsession with weight loss on its head. Losing the gut is always a good idea—the less visceral fat the better. But what if dropping the wrong kind of pounds—from the hips, for example—could make us less metabolically fit, if we lose precious beige fat cells in the process? The message from Cohen is that having an overweight, pear-shaped body isn't all that bad. It may even be healthy.

"Beige fat cells may not promote weight loss so much," Cohen says, "but they promote metabolic health, and that's fine. At the end of the day, for our vanity, we might like to get back to the weight we had when we finished high school, but our health is much more important than what the scale says." ©

Tracking time isn't something we do with just our brains and our wrists. Most cells in the human body can mark the passage of Earth's 24-hour rotation. Meet the scientist devoted to the biology of the day.

Michael W. Young

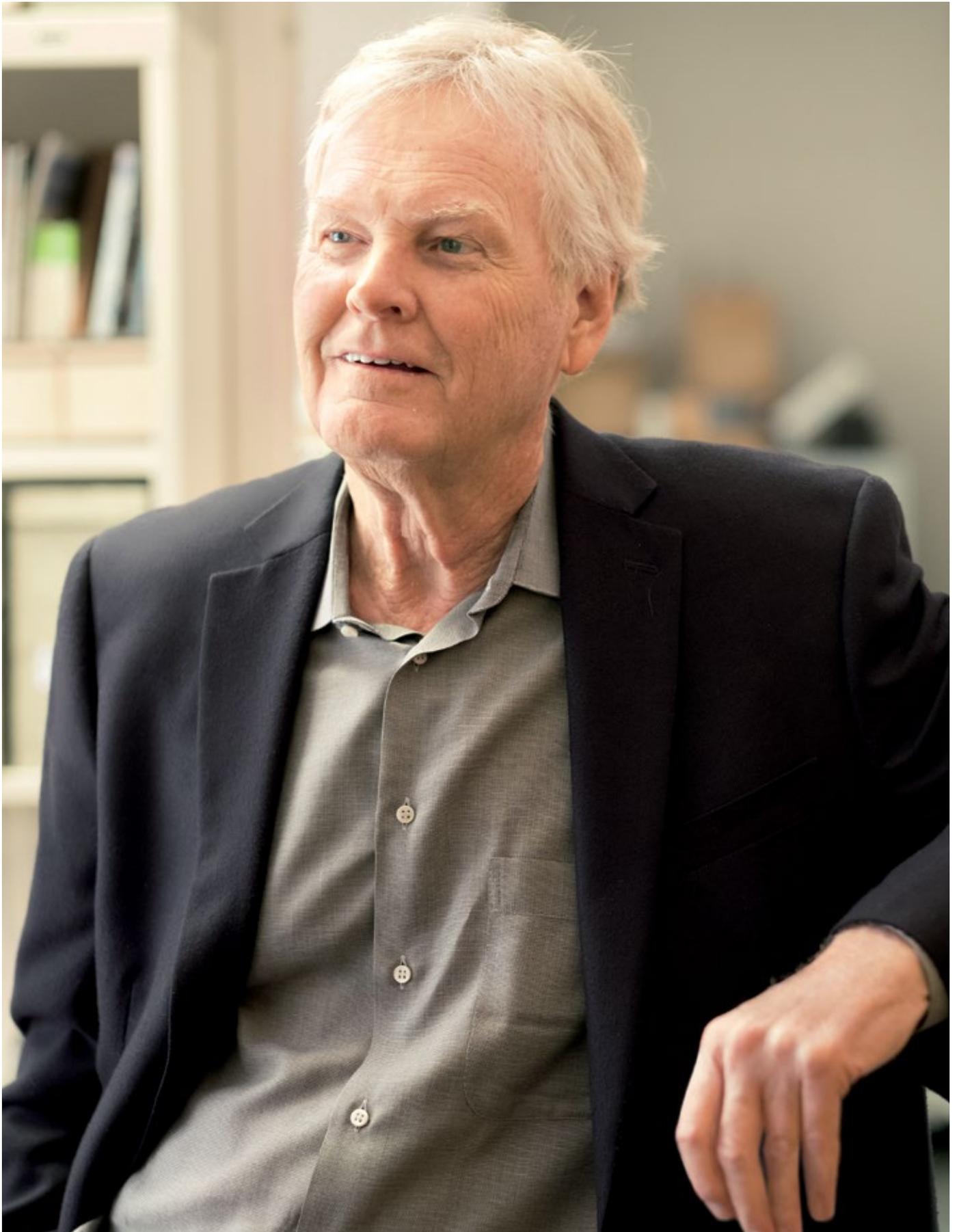
By Zachary Veilleux

It's easy to tell if a fruit fly is awake or asleep. If it's flying around, it's awake. If not, it's dreaming of spoiled mango.

When Michael W. Young began working on circadian rhythms in the early 1980s, he needed some way to automatically record his flies' sleep-wake cycles. Rockefeller's in-house machine shop built him a custom tool: a breadbox-sized piece of equipment capable of monitoring five flies at a time, causing a signal every time one of its occupants ran past a sensor. Active flies would zip back and forth endlessly; those that didn't trigger the device were asleep.

It was a simple concept, but it proved indispensable. Over the years, Young and his lab members put thousands of genetically altered flies in the machine's tiny glass tubes (and those of its successors), and the results led to discoveries of the key genes and proteins all organisms use to regulate their internal clocks—discoveries for which Young received the Nobel Prize in Physiology or Medicine this year.

We spoke to Young, who is the *Richard and Jeanne Fisher Professor*, about his favorite biological mechanism a few days after the prize was announced.



Why did you decide to study clock genes?

I didn't at first. Originally I was a *Drosophila* geneticist, looking more generally at how genes of animals might differ from those of bacteria. I was also interested in transposable elements—segments of DNA that jump from one location on the genome to another—and trying to identify what role they had in the life of a fly. On the way I became interested in a gene, called *period*, that was related to circadian rhythms.

I had first come across the *period* mutations as a graduate student, and thought it was something I might like to work on. The more I looked at it, the more it revealed itself to be a very pretty piece of biology. It had a lot of mystery, and the ramifications turned out to be far beyond what we imagined.

So you narrowed your focus?

We dropped the other work and tried to isolate the *period* gene. That's when we built the sleep-wake recorder, so the postdocs wouldn't have to stay up all night with a stopwatch, watching flies walk and sleep. We used it on mutant flies that were arrhythmic—they had no regularity to their sleep patterns—and inserted DNA into them that we thought contained the *period* gene. Every morning we'd come in and read the printouts logging their sleep patterns. In those experiments we were able to restore rhythms to arrhythmic flies, proving we had our gene.

An important early finding was when we found a translocation in the genome. We saw that a snippet of DNA breaks from the tip of the X chromosome and integrates itself into the fourth chromosome. Flies with this translocation would have no discernable pattern of sleep. This was our earliest indication of where exactly the gene was in the chromosome we were studying, and we eventually found that this translocation was breaking a non-coding part of the *period* gene—meaning this was a problem not of the gene itself, but of its regulation. That was a big clue.

What's the most important thing you've learned about how these cycles work?

This is a story not of a single big discovery, but of the accumulation of small discoveries over many years that eventually led to a fairly clear picture of how cells track time.

Besides the *period* gene, we found several other genes involved in this system. Eventually we discovered that *period* works with a gene called *timeless*. The protein products of these two genes accumulate together and pair up in the cell's cytoplasm. When a certain threshold is reached, they migrate into the nucleus where their presence stops further production of the two proteins by shutting down the corresponding genes.

It then takes a while until the proteins clear out and the brake on their genes is released—at which point a new cycle begins. This cycle of accumulation—driven by a few other genes that we have identified more recently—generates a consistent delay and sets up a daily rhythm.

Finding *timeless* was the key, but it wasn't easy. There was a rule of thumb in genetics at the time that if you can't find what you're looking for in the first 200 strains, it's not there. And we'd done that. So we decided to go past 200, and we in fact had to search 7,000. We decided the 200 rule was an absurdity.

Why do all our cells need to know what time it is? Why isn't that something that the brain simply keeps track of?

So much of our biology depends on what time it is, and having seamless coordination between different tissues is critical. This means each organ needs to do its job at the right time without having to wait for another organ, like the brain, to tell it what to do.

For example, firing up the body from a sleeping state to one where we can function requires time-dependent regulation across different tissues. Things like blood pressure, growth hormone, cortisol, heart function, muscle strength—all are changing over the

“There was a rule of thumb that if you can't find what you're looking for in the first 200 strains, it's not there. We in fact had to search 7,000.”



Drosophila nap tracker

A custom device to record flies' sleep patterns, built to Young's specifications in the early 1980s, was an essential tool for pioneering research on clock genes. Capable of accommodating five flies at a time in glass chambers, it used photoreceptors and tiny LEDs to generate a signal each time an awake fly walked past. An attached ink printer output this activity on long rolls of graph paper, allowing scientists to easily log when their subjects fell asleep and woke up.

Today the lab uses modern, off-the-shelf versions of the device capable of monitoring hundreds of insects simultaneously and logging the data to computers. But the underlying concept hasn't changed.



Reporters visit Young's lab following the October 2 Nobel Prize announcement.

produced by this gene, you could eliminate it from the system. Every cell would lose the dominant influence of that variant and presumably be restored to normal function.

But in general, we see a lot of genetic variability among people with sleep problems. In many cases, the ability to have an effective therapy will be very much dependent on whether there is a genetic change and, if so, which mutation you have.

Has what you've learned in fruit flies translated nicely into people?

Much more so than I would ever have thought at the outset of this work. Fly clocks and human clocks, it turns out, mostly run on the same genes and proteins, and even when there's a variation it typically doesn't change the way the whole system works.

For the past several years we've actually been doing clinical work to study the effect of various gene mutations in volunteers. Thanks to vast databases I can sit at a computer and look at every gene I know contributes to how these clocks run. I can see what kinds of mutations have been found, and I can make initial guesses about what might cause shifts in sleep-wake cycles.

We can then test those mutations in cellular systems and ask how frequently they are seen in the population. If it's frequent, we have a good chance of being able to find people who will volunteer for our studies—and we have an idea what to look for.

How will the Nobel Prize affect work in this field?

My hope is that people will begin to talk about circadian rhythms with a level of understanding they already have for things like DNA. A Nobel Prize can draw attention to this field, in which so much growth has occurred in recent years, and translate it to something that everybody thinks they should know something about. This work is not hard to grasp, and the implications are very broad.

Circadian rhythms are a major component of our biology—we are rhythmic organisms. ©

course of the day. Some are changing more in anticipation of the cycle of daylight and temperature, but others are cued to food. This allows for critical body functions to be reproducible, and it gives you the ability to be ready for recurring daily events—like meals—at the right time.

What happens when we colonize another planet where the days are longer or shorter than 24 hours? Will we adapt?

Initially we won't. We'll live in perpetual jet lag, or else we'll create an artificial environment to mimic the rhythm we are used to. But eventually, as a species, we will evolve. We have faced this problem before: the days were shorter hundreds of millions of years ago, when Earth rotated at a higher rate.

One of the things our research has shown is just how easy it is to manipulate these clocks. By changing just a couple of letters in the DNA code, we can create a fly with a four-hour change in periodicity. We've made flies with clocks ranging from 16- to 40-hour cycles that run just as well as the regular, 24-hour clock. When the time comes, evolution will be ready.

One could imagine a pill that would allow people to function on less sleep, to move to a 40-hour cycle so they could be more productive. Is that realistic?

The problem is you would need to move the clocks running in each of your tissues all in the same direction and at the same rate. And it's hard to devise a single medication that can accomplish that. If they get out of sync it's like jet lag: you might be able to adjust your sleep schedule relatively quickly, but you'll still get hungry at the wrong time. These timekeeping systems can have a lot of inertia, and some organs require more reinforcement than others before they adjust.

What about devising a cycle-restoring medication to help people with sleep disorders?

There is some potential here. One thing we've found, looking at people who have a syndrome called delayed sleep phase disorder, in which bedtimes are consistently shifted two to three hours, is a common mutation that produces a hyperactive protein and is present in the clock of every cell. If you create a medicine that interacts specifically with the version of the protein

Live-cell imaging in your pocket

SOMETIMES THE VIEW through a microscope is too good to keep to yourself.

Fortunately, it turns out that smart phones, with their integrated cameras and high-resolution screens, make pretty good devices for sharing microscope images—all you need is the right adapter to align the optics.

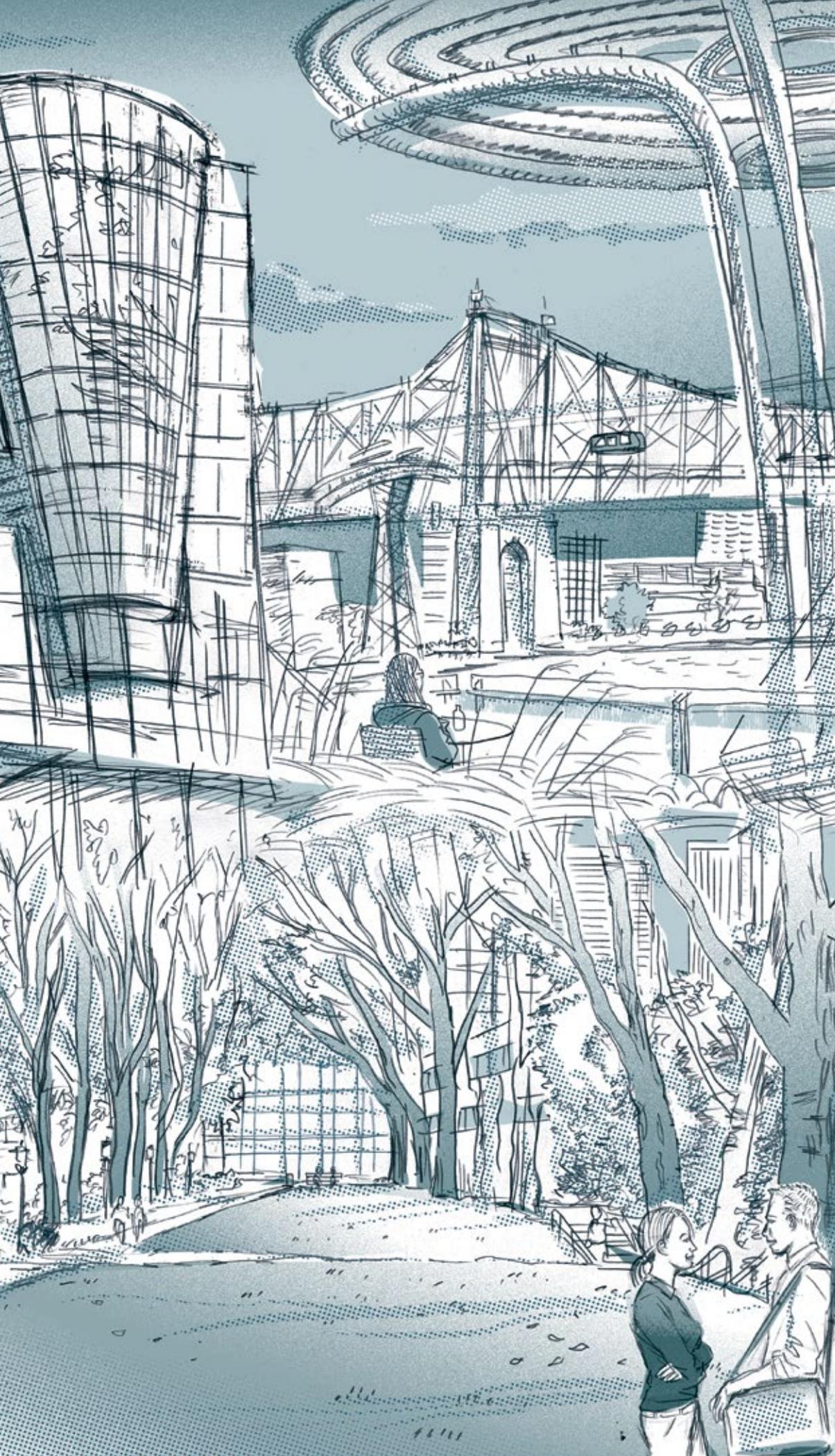
Du Cheng, a Rockefeller M.D.-Ph.D. student, has created just such an adapter. He made his first prototype out of styrofoam while still an undergraduate. At the time, it was a struggle to work the awkward bolt-on cameras attached to classroom microscopes.

Since then his devices, which he calls LabCams, have evolved into molded plastic shells with inte-

grated eyepieces, custom-designed for several models of iPhone. The contraption can dock to nearly any microscope. Once in place, the phone's camera takes over, providing crystal-clear, recordable views of worms, flies, bacteria, tissue samples, or any other diminutive object a biologist might be interested in. No squinting required.

Cheng and his labmates rely on the LabCam to document the behavior of the tiny nematode worm *C. elegans*, but a growing community of users on and off campus have captured images of everything from stem cells to rat sperm. They're even co-opting apps like FaceTime to share findings in real time. ☉





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