ISSUE
09
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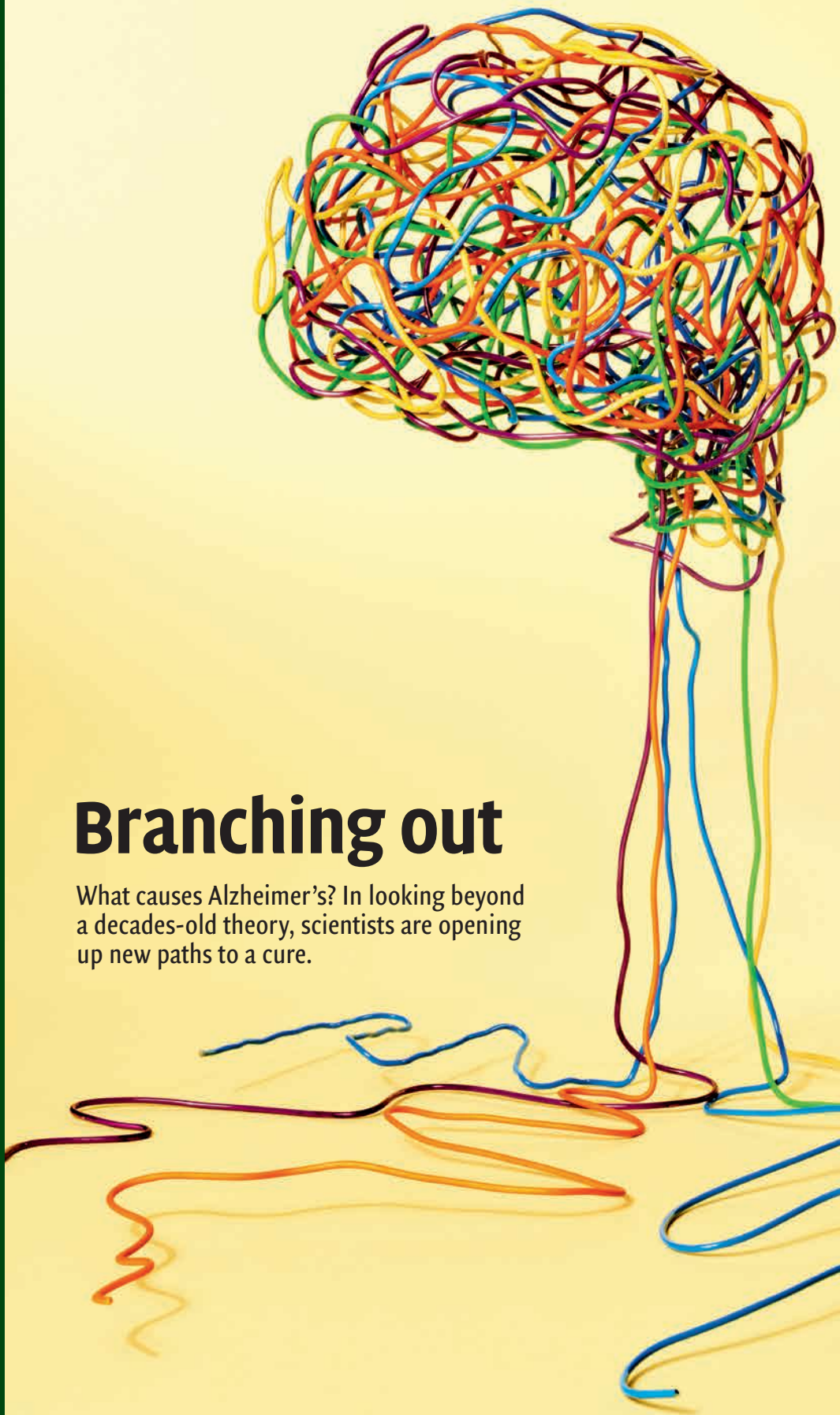
2023

Seek

THE ROCKEFELLER UNIVERSITY



Branching out

What causes Alzheimer's? In looking beyond a decades-old theory, scientists are opening up new paths to a cure.

ALSO

"We aren't saying that plaques aren't involved. We're saying that targeting plaques is not the only approach for treating this disease."

18

New leads, new hope

The idea that Alzheimer's is caused by brain plaques has long underpinned efforts to treat it—with mostly disappointing results. Now, a growing movement among scientists is challenging basic assumptions about the disease and uncovering fresh clues across multiple biological systems. Is it the start of a new chapter?

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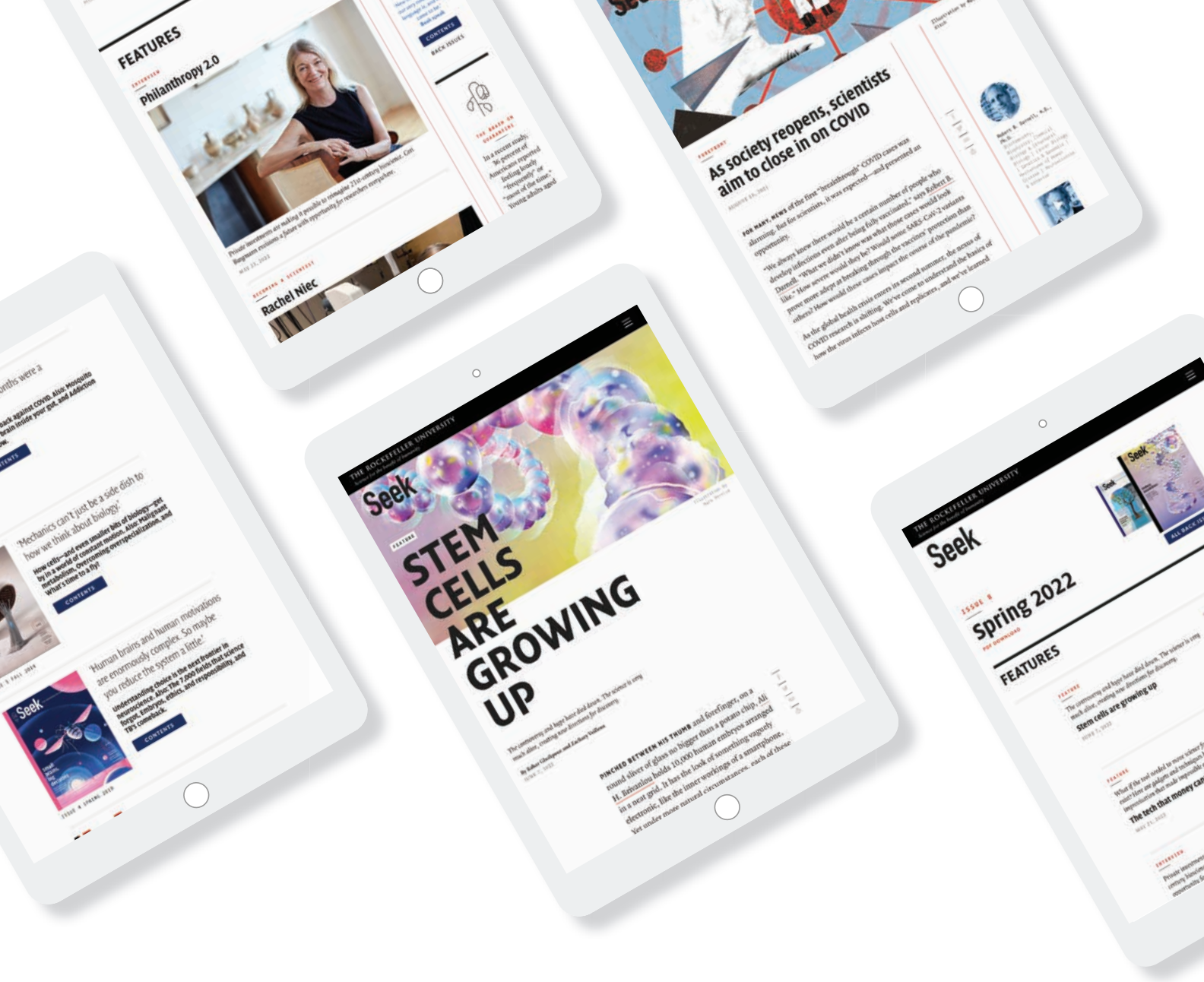

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CONTENTS

ISSUE 09 WINTER 2023

"In a manner of speaking, the brain is constantly hallucinating."

Illustration by Saiman Chow

FEATURES

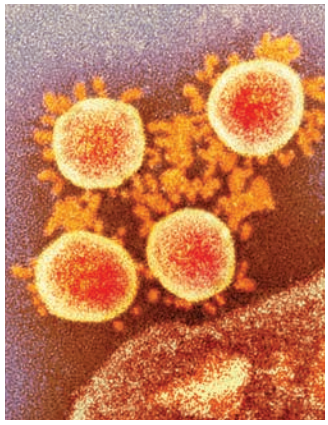
36

Is the key to COVID in our genes?

Scientists from around the world are on a quest to understand why SARS-CoV-2 makes some people sicker than others. Their approach is changing how we think about COVID or any infectious disease.

Of all the unknowns surrounding this disease, the most confounding is how and when it starts.

PAGE 22



42

Talking about a revolution

Cryo-electron microscopy has supercharged the study of life's finest details. For Jue Chen, it has opened up new worlds of discovery.

14

Q&A

Vaccines give us lifetime protection us against polio, measles, and smallpox. Why can't they do the same for COVID, HIV, and the flu? Biologist Pamela J. Bjorkman has a promising new strategy.

FOREFRONT

6

Are flies good at math? What happens in the brain when we're sick? **And why are woodpeckers drumming up excitement among evolutionary biologists?**

ON THE BACK COVER
The Rockefeller University
by Sally Deng

ZACHARY VEILLEUX; CLAIRE HOLT; NATIONAL INFECTION SERVICE / SCIENCE PHOTO LIBRARY; ISTOCK / LEON GIN

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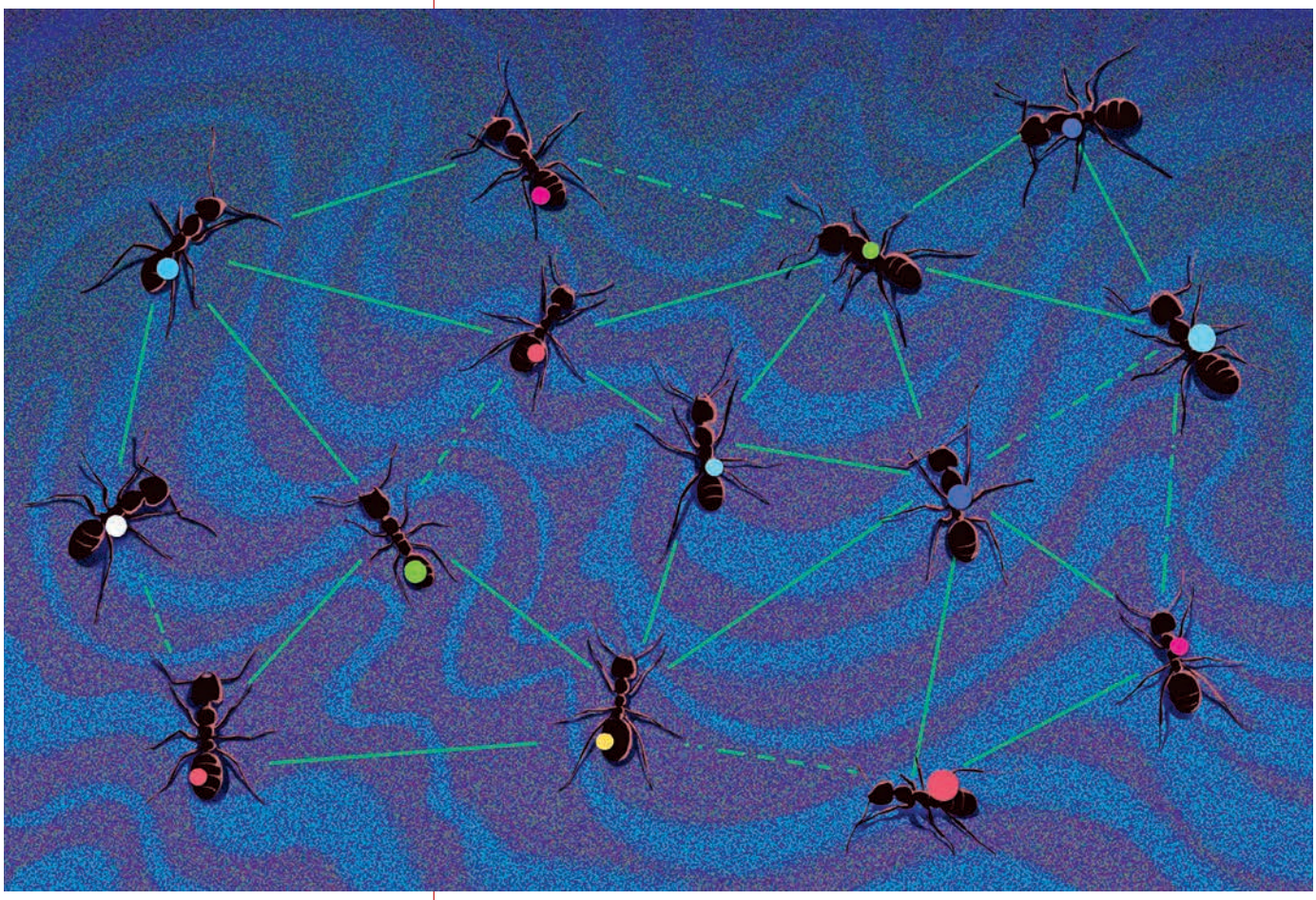
A fuller picture. Thirty-five portraits line Rockefeller's halls, most showcasing men scientists. The recently unveiled 36th tells a more inclusive story, highlighting five trailblazing women scientists. From left, there's Florence Sabin, who advanced understanding of the immune system's response to tuberculosis; Louise Pearce, creator of the first effective treatment for African sleeping sickness; Rebecca Lancefield, at the center, classified subtypes of strep bacteria, then ravaging WWI battlefields; next, Gertrude Perlmann deciphered pepsin's 3D shape and its role in digestive disorders; Marie Daly, the first Black woman in the U.S. to receive a Ph.D. in chemistry, was instrumental in linking hypertension and high cholesterol to an increased risk of heart attack.

Photo by Will Ragozzino

Reported by Lori Chertoff, Mindy Farabee, Bahar Gholipour, Eva Kiesler, Joshua Krisch, and Jen Pinkowski.

FOREFRONT



SOCIAL LIFE

When all think as one

SINGLE ANTS RARELY make headlines, but ant colonies are capable of incredible things. Watch them transform a pile of dirt into an elaborate ant hotel, for example, and you might think that a single mind is steering the entire colony. In fact, this may not be far from the truth, according to recent work from Daniel Kronauer's Laboratory of Social Evolution and Behavior.

The researchers found that ants can behave in much the same way that neurons collaborate in the brain, with regard to a process called sensory thresholding. Common to virtually all animals, sensory thresholding is a kind of cost-benefit analysis in which the organism reacts to a sensory input only when that stimulus crosses a certain threshold.

For instance, sensory thresholding may be at play when you decide to move out of a hot room. The point at which you'll get up and leave will depend partly on the rising temperature and partly on internal factors, like the body's need to preserve energy. You may initially stay put, but once the room gets hot enough to justify the hassle, you'll head for the door.

Kronauer and postdoctoral associate Asaf Gal wondered if ants would engage in sensory thresholding as a group, similar to the way neurons do in a brain—putting the needs of the whole network over that of individual cells. Working with clonal raider ants, Kronauer and Gal marked each ant with color-coded dots, let them form a nest, turned up the heat of the nest in precise increments, and tracked the ants' responses.

Illustration by Tianhua Mao

Predictably, the ants fled the nest when temperatures reached uncomfortable levels, but they behaved more like a neural network than like humans shuffling out of a room. Specifically, how hot the nest had to become before the insects made an antline for the exit depended on the size of the colony: Those with around 50 members consistently fled at around 34 degrees Celsius, while colonies of 200 held out until the temperature reached 36 degrees.

This phenomenon is hard to explain if you think of ants as isolated individuals—an ant doesn't know how many peers it lives with, so how can its decision depend on colony size? Kronauer and Gal suspect that pheromones, the messengers passing information between ants, scale their effect when more ants are present.

Still, why larger colonies require higher temperatures to pack up shop remains unclear. "It could simply be that the larger the colony, the more onerous it is to relocate, pushing up the critical temperature for which relocations happen," ventures Kronauer, Rockefeller's *Stanley S. and Sydney R. Shuman Associate Professor*.

The findings, reported in *Proceedings of the National Academy of Sciences*, suggest that ants combine sensory information with the parameters of their collective to arrive at a group response. And according to Kronauer, the research is "one of the first steps toward really understanding how insect societies engage in collective computation." ◎

JACOB PRITCHARD

MEDICAL MYSTERIES

We need our fat

THERE'S MORE TO fat than meets the eye. We tend to think of our adipose tissue as being as unneeded as it is unwanted, nothing more than the much-maligned result of eating more calories than we burn. But scientists have come to realize that fat isn't just a byproduct of metabolism; it's also one of the key places where it happens. In fact, it would be better to think of fat tissue not as padding but as a complex, full body organ—with its own constellation of immune cells and nerve projections—that's in constant dialogue with the endocrine system.

We need our fat, just like we need our intestines, liver, and stomach. And just like any organ, fat can suffer damage, with serious consequences.

Paul Cohen, a physician-scientist on staff at Memorial Sloan Kettering Cancer Center who studies obesity and its comorbidities, began to suspect that fat might be involved when survivors of childhood cancer showed up in his office with cardiometabolic diseases.

"I kept returning to this distinct group of patients," he says. "They were developing coronary heart disease or diabetes at younger ages than expected in the absence of typical risk factors like obesity."

Though Cohen's patients presented as seemingly healthy young adults, all with normal BMIs and waist-to-hip ratios, they were already displaying the subtle indicators of brewing metabolic disease, such as rising blood sugar. Moreover, their fat tissue was brimming with immune cells and proteins known to be elevated in response to chronic injury.

And Cohen's lab identified another commonality among these cancer survivors: As kids, they had all been treated with abdominal or total body irradiation. His hypothesis: Early exposure to radiation may cause long-term dysfunction in fat cells that manifests decades later.

He hopes that these findings, published recently in *JCI Insight*, will make clinicians rethink what they think they know about our metabolic systems. "When physicians are planning radiation therapy, they are very conscious of avoiding damage to major organs," says Cohen, the *Albert Resnick, M.D. Associate Professor* at Rockefeller. "But fat is often not considered." ◎

A blob of fat is as worthy of investigation as any other tissue.

A memorable walk in VR

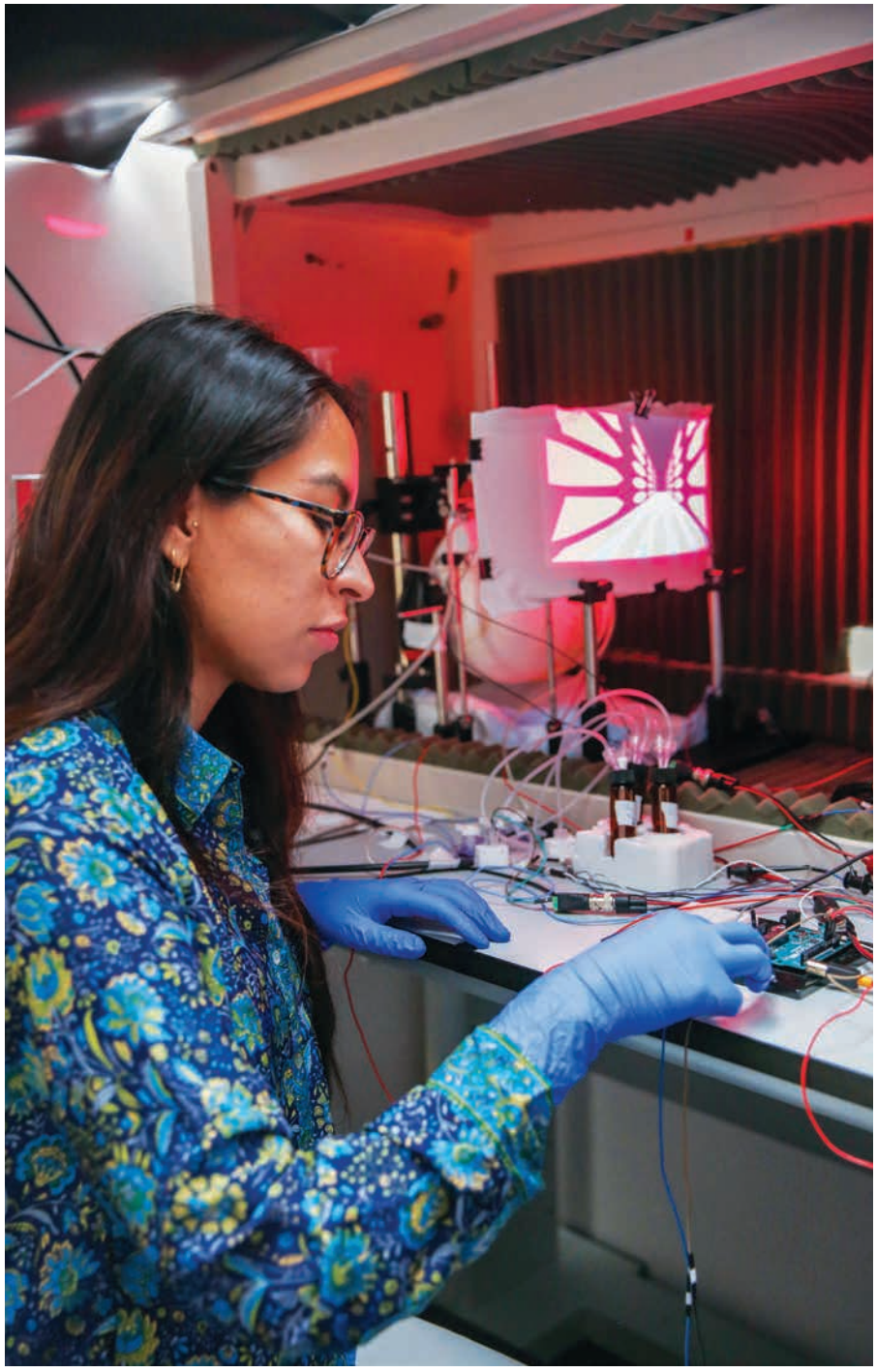
NEUROSCIENTISTS HAVE LONG known that memories are formed in the hippocampus, a small structure at the core of the brain. But recent findings suggest that's only part of the story.

Researchers in the lab of Priya Rajasethupathy, the *Jonathan M. Nelson Family Assistant Professor*, found that a complex memory consists of a whole and its various details. You may bring to mind the full experience of last night's dinner outing, for example, or just the taste of that ragù or a glimpse of candlelight. Working with mice, the scientists found that while the whole memory is stored right where they expected it—in the hippocampus—the fragments unexpectedly popped up in the prefrontal cortex.

To arrive at these discoveries, the researchers had to jump a few hurdles. Technical limitations have long hampered efforts to study memory as a distributed brain process. So Nakul Yadav, a graduate student in Rajasethupathy's lab, built a novel setup using virtual reality to simultaneously record and manipulate neural activity from multiple brain areas. Perched atop a rolling Styrofoam ball, mice in the experiments strolled down an endless VR corridor, encountering various multisensory experiences along the way, each with its own pattern of lights, sounds, and smells. These sensory cues trained them to associate different "rooms" with pleasant or less-than-pleasant experiences. Nudged later by a specific sight or scent, the mice were able to recall the broader context and knew whether to happily expect sugar water or look out for an annoying puff of air.

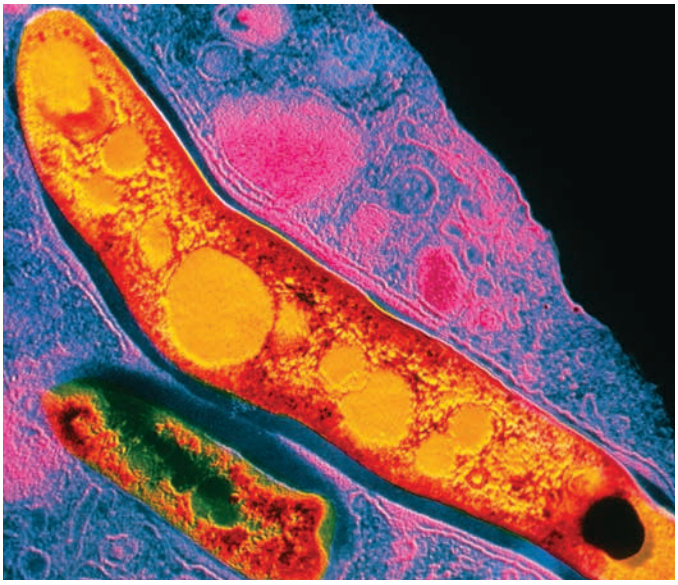
In the process, the researchers discovered that a particular neural circuit makes two brain regions work in tandem during memory recall. This circuit lights up when prompted by the right kind of sensory input—a smell you come to associate with the meal, for example—and activates the prefrontal cortex, which then accesses the hippocampus for full memory retrieval. The work points to a new understanding of how the brain processes a memory.

"It suggests that there's a dedicated pathway for memory recall, separate from memory formation," says Andrea Terceros, a coauthor of the study the team published in *Nature*. The findings might ultimately inform the treatment of dementias such as Alzheimer's, which may be less about deficient memory storage than a breakdown in memory recall.



Terceros at the VR station where mice create new memories.

JOHN ABBOTT



CNRI

PANDEMICS

TB's mutation mistake

IN SOUTHEAST ASIA, one particularly virulent tuberculosis strain infects half a million people each year. There may soon be a simple solution to that problem: macrolide antibiotics.

True, macrolides have historically failed to treat TB. But if the bacteria were to mutate in a particular way, TB would crumble in the face of these FDA-approved drugs—and in Southeast Asia, that's exactly what appears to have happened. Jeremy M. Rock and colleagues serendipitously discovered that the Southeast Asian strain picked up precisely the right mutation about 900 years ago—rendering it vulnerable, in theory, to a class of readily available drugs. Since publishing findings in *Nature Microbiology*, Rock, who is Rockefeller's *Penrhyn E. Cook Assistant Professor*, has been devising a way to apply them in a clinical setting, an effort that could ultimately save thousands of lives. ◎

DATA

1952

The year when the original macrolide antibiotic, erythromycin, was first used. There are now three FDA-approved drugs of this class.

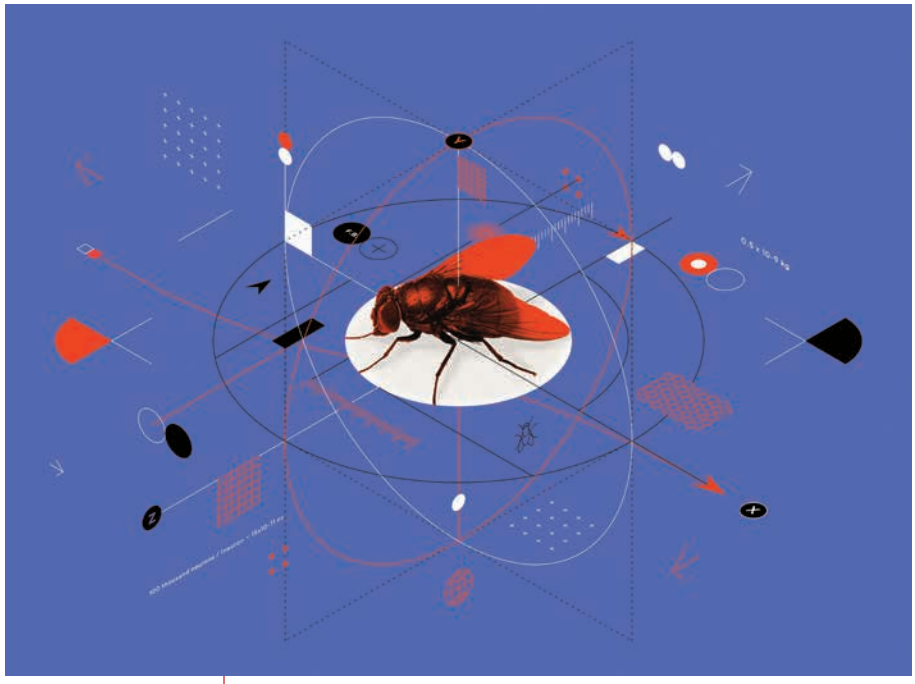
SPATIAL STRATEGIES

Math on the fly

YOU'RE WALKING DOWN the street on a sunny autumn day. You hear a bang and, without skipping a beat, turn your head to see what's going on: Someone slammed a car door. Sound identified, you march on.

We usually take for granted our ability to walk in one direction while facing another without getting disoriented. But perhaps we shouldn't; in the lab of Rockefeller's Gaby Maimon, scientists are fascinated by the brain's ability to construct a sense of spatial orientation as we move through the world.

Recently, Maimon and graduate student Cheng Lyu discovered a set of math-savvy neurons in fruit flies that might reveal how the animals keep heading in the right direction. These neurons, of which there are four classes, perform complex mathematical calculations along four axes to indicate the fly's traveling direction. In work reported in *Nature*, the researchers found that each neuronal class can be thought of as representing a mathematical vector whose angle points in the direction of its associated axis. A vector's length indicates how fast the fly is moving in that direction.



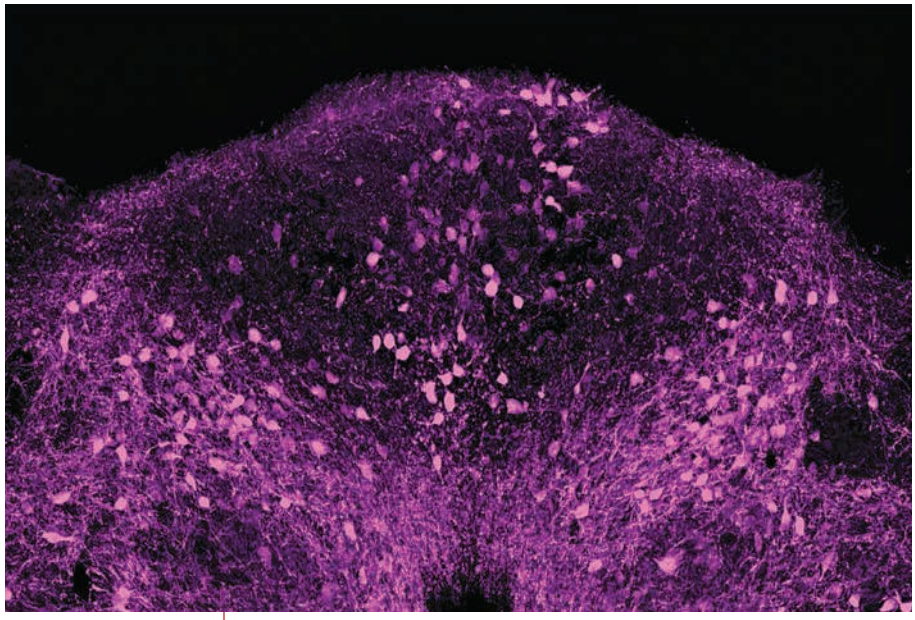
Vector math is integral to the brain's navigational functioning.

Illustration by Israel G. Vargas

"Amazingly, a neural circuit in the fly brain rotates these four vectors so that they are aligned properly to the angle of the sun and then adds them up," Maimon says. "Neuronal circuits implement relatively sophisticated mathematical operations." ◎

RECUPERATION

These cells light up when you're down for the count



These neurons provide clues to what the brain is up to when the body fights a pathogen.

IT'S EASY TO relate to a mouse feeling under the weather. It seeks out a safe spot to hide, preferring not to move, and it doesn't have much of an appetite.

In fact, most animals will avoid moving, eating, and drinking when they're fighting an infection. It's the smart thing to do—these near-universal sickness responses allow the organism to save energy that the immune system needs to fight off the pathogen. But precisely how the behaviors are orchestrated was an open question when researchers in the lab of Jeffrey M. Friedman, the *Marilyn M. Simpson Professor*, set out to search for neural activity induced by an immune response.

A cluster of neurons lit up in the brain stem whenever the scientists provoked a mouse's immunity. When firing, these cells subdued the animal's movement, eating, and drinking; and when the researchers directly triggered the same neurons in healthy mice, those animals began displaying similar behaviors. The findings were published in *Nature* in October.

Anoj Ilanges, a former graduate student in Friedman's lab who is now a group leader at the Howard Hughes Medical Institute's Janelia Research Campus, says that little is known about the central nervous system's role in infection. "We looked at one region of the brain," he says, "but there are many others that become activated with the immune response. This opens the door to asking what the brain is doing holistically during sickness." ◎

ANIMAL KINGDOM

New blood in the copycat club

PRECIOUS FEW ANIMALS can learn to imitate new sounds, a skill known as advanced vocal learning. Humans and parrots can do it, as can whales, seals, bats, hummingbirds, songbirds, and elephants, the last of which have been observed copying the sounds of passing trucks. New research from the laboratory of Erich D. Jarvis welcomes woodpeckers as members of this rather exclusive club, albeit for a different reason.

Jarvis and his team were surprised to discover that woodpeckers possess specialized neural circuits that resemble the brain structures that allow young songbirds to learn new tunes. However, the woodpecker brain regions were activated not by vocalization but by drumming on tree trunks—a rhythmic behavior that the birds use to compete for territory. Writing in *PLOS Biology*, the team proposed that the woodpecker drumming circuit and the vocal learning pathways of songbirds and humans evolved from the same ancestral structure. ◎

LABORATORY OF MOLECULAR GENETICS; ISTOCK / LEON GIN

Pfizer, Moderna, Johnson & Johnson, AstraZeneca, Gamaleya, CanSino, or Sinovac?

198

DATA

Number of doses of COVID vaccine administered per 100 people in the United States. In Mexico, that number is 174; in Cuba, it's 335.

Population immunity might be achieved even without the best vaccines.

ISTOCK / MARSBARS

COVID

All vaccines on deck

"DID YOU GET Pfizer or Moderna?"

Here in the United States, this question became something of an icebreaker. But in Mexico, where officials administered seven different COVID vaccines, it would not have rolled off the tongue quite so easily: Pfizer, Moderna, Johnson & Johnson, AstraZeneca, Gamaleya, CanSino, or Sinovac?

"Mexico allowed concomitant use of different vaccines, including those not yet approved by the World Health Organization, since there was insufficient vaccine production to meet the demand," says Santiago Avila-Rios, an assistant professor at the National Institute of Respiratory Diseases in Mexico.

To what extent this motley approach to population immunity worked had been unknown, however, since the vast majority of research on vaccine efficacy has been done on the big-name mRNA vaccines, Pfizer and Moderna. So Rockefeller virologists Theodora Hatziioannou and Paul Bieniasz teamed up with Avila-Rios and other researchers in Mexico to find out.

"They were using many vaccines that we haven't seen in the U.S.," Hatziioannou says.

There were clear concerns that the lesser-known vaccines would offer inferior protection compared with big names like Pfizer and Moderna, yet the researchers didn't discount the possibility that some of those unsung vaccines might actually work better. "Some vaccines involved different methods of inducing immunity—adenovirus vectors, whole activated viruses, multiple doses," Hatziioannou says. "So we wanted to know what level of neutralizing antibodies were achieved with these other methods."

As it turned out, the vaccines elicited a range of immune responses: Pfizer was the strongest; Sinovac, the weakest. None provided much neutralizing activity against the omicron variant in patients who had never been exposed to the virus, but all the vaccines produced neutralizing antibodies against omicron in those who had been infected either before or after vaccination.

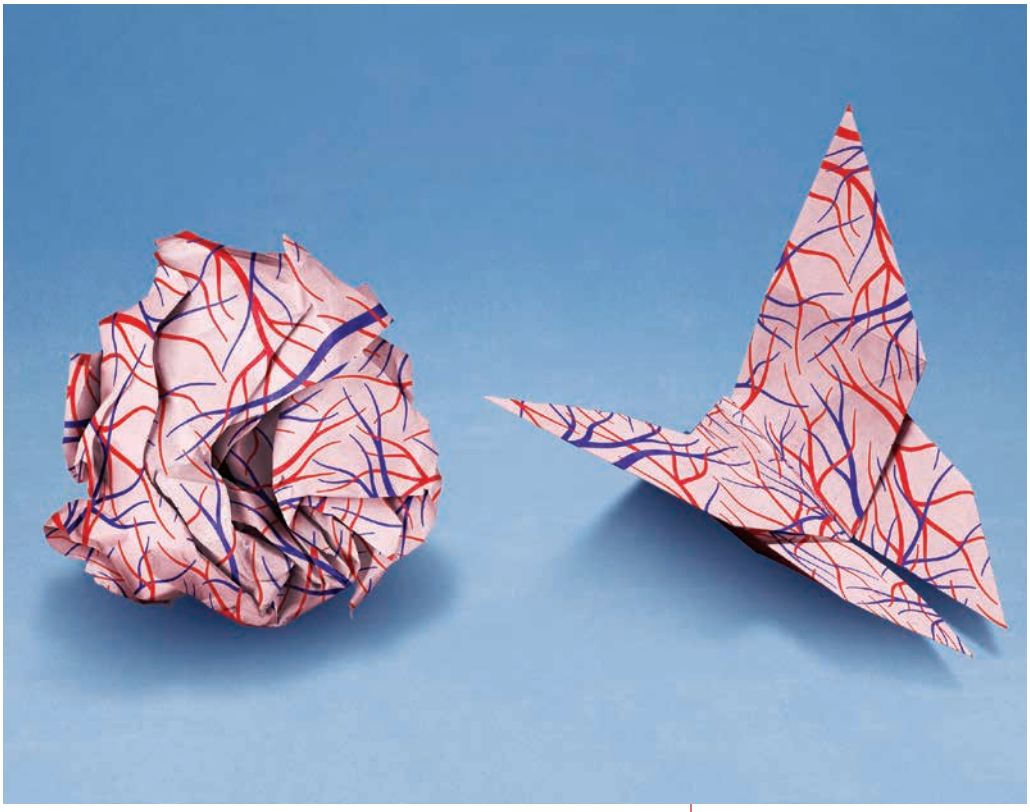
The results were heartening at the population level, where the data suggests that low- and middle-income countries will be able to achieve immunity by hook or by crook—by vaccinating and boosting individuals with whichever vaccines are available, or through repeated exposure via infection. "Even in the absence of the best vaccines, it might be possible to achieve population immunity with a mix of approaches," Hatziioannou says about the study, published last year in the journal *mBio*. "We expect that low- and middle-income countries will ultimately achieve good levels of immunity against the virus, though they might need to prioritize boosters for people who received vaccines that elicit low levels of antibodies." ◎

OMICRON

The benefit of boosters

OMICRON VARIANTS HAVE been stalking the globe for over a year, and they're wildly infectious. If you're overdue for a booster, now's the time to get one. A team led by Paul Bieniasz, Theodora Hatziioannou, and Michel C. Nussenzweig reviewed blood samples from individuals who had received second and third doses of an mRNA vaccine, and they found up to 200-fold increases in neutralizing activity against the omicron variant. They reported their results in the *New England Journal of Medicine*.

"Our study makes it clear why the third dose should be recommended," says Nussenzweig, the *Zanvil A. Cohn and Ralph M. Steinman Professor*. "It's one of the best reactions to the virus that we've seen." ◎



AT ATOMIC RESOLUTION

How misfolded proteins get into shape

THE PROTEINS IN our bodies are constantly twisting themselves into the most exquisite origami. Flipping and folding just so, strings of amino acids take on precisely the right forms—pleats, horseshoes, jelly rolls—to keep our functions beautifully humming along. Except when they don't—and one misshapen molecule can spell catastrophic system failure.

Cystic fibrosis is a prime example. Doctors have long understood exactly how the disease does its devastating work: At its heart is CFTR, a protein channel lying atop cells lining the lungs and digestive tract that attracts water to thin and move mucus. But if CFTR doesn't fold correctly, it can barely function at all. Then mucus accumulates and hardens, breathing and digestion become painfully difficult, and the lungs become a fertile ground for pathogens.

Fortunately, powerful drugs called correctors can significantly prolong patients' lives. Until recently, no one truly understood how they worked—until Jue Chen, Rockefeller's *William E. Ford Professor*, and her team demonstrated how one medication known as a CFTR corrector directly addresses the misfolding.

They did it by stitching together thousands of snapshots of the corrector in action, developing a clear picture of how the drugs stabilize CFTR by nestling into a notch within the protein. That understanding enabled them to develop a theory, published last year in *Cell* and *Science*, of how protein folding correctors do their job. As it turns out, Chen's group may have opened the door to treatments for a range of heretofore intractable conditions.

Hundreds of diseases, from Parkinson's to sickle cell anemia, occur when proteins fail to assume the correct 3D structure. "We now have a way to identify molecules that may be used to treat these diseases," Chen says. ◎

DATA

Approximately 2,500 mutations in the CFTR gene have been linked to cystic fibrosis.

Illustration by Matt Chase

DISORGANIZED DNA

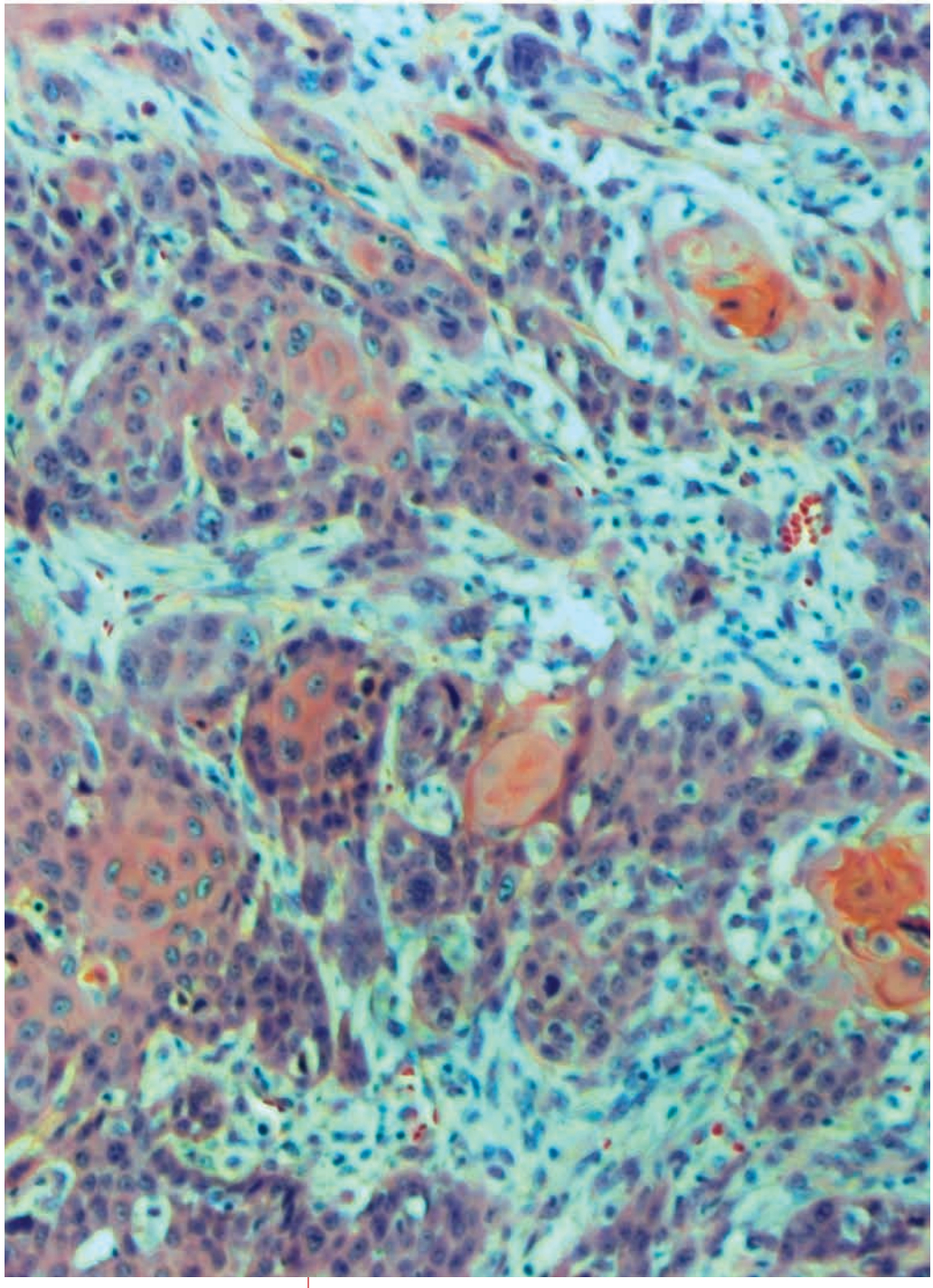
Two diseases, one problem

SCIENTISTS HAVE FOUND an intriguing relationship between patients with Fanconi anemia, a rare genetic disorder, and people without the disease who smoke cigarettes: Both are vulnerable to a certain kind of genetic havoc, increasing their risk for developing head and neck squamous cell carcinoma, a common cancer growing in the mucous membranes of the mouth, nose, and throat.

In work published in *Nature* in November, the lab of Agata Smogorzewska found that Fanconi patients' cells are unable to repair DNA damage caused by chemicals called aldehydes found in some foods and in cigarettes. As a result, their genomes acquire structural problems—a sort of Goldilocks syndrome in which genes are present in too many or too few copies, with stretches of DNA appearing in the wrong places or not at all, creating a perfect storm for the development of quickly metastasizing tumors.

As it turns out, similar genomic defects are observed in tumors from people without Fanconi anemia, and the researchers found a correlation between these individuals' smoking history and the frequency of structural variants: The more a person had smoked, the more variants were detected in their tumors. Smogorzewska posits that smoking subjects the body to so much aldehyde-induced damage that otherwise healthy repair mechanisms fail to keep up.

"So, cells from people without Fanconi anemia act as if they too have a DNA-repair defect," she says. "This rare disorder may be telling us something profound about how certain cancers are triggered in the general population." ◎



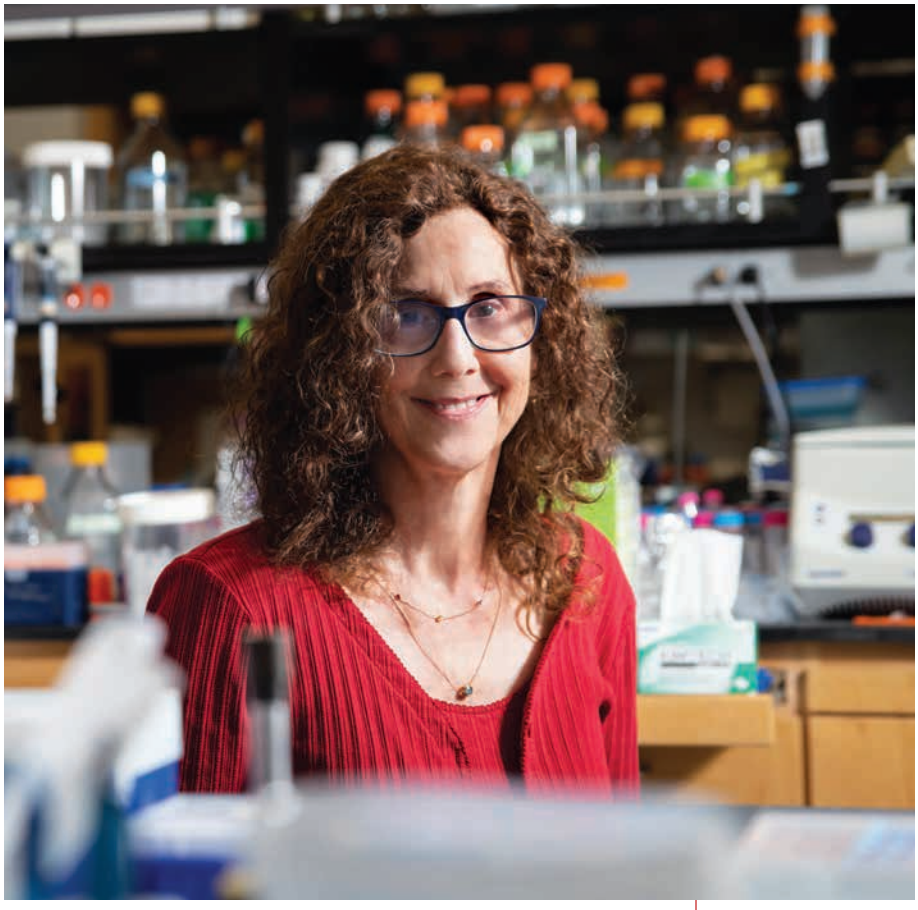
At the molecular level, tumors of heavy smokers look a lot like those of patients with Fanconi anemia, pictured.

LABORATORY OF GENOME MAINTENANCE

Q&A

How to end a pandemic in one jab

With Pamela J. Bjorkman



Bjorkman wants to teach the immune system to see coronaviruses differently.

SARS-COV-2 IS A wily shape-shifter. It mutates into new forms so frequently and expertly that even though scientists have reformulated vaccines in record time, we've already fallen behind. The arrival of a universal vaccine—one that could provide long-lasting protection against every variant—would signal a momentous turning point in the pandemic, letting us breathe easy despite the looming specter of even more virulent strains.

It's not impossible. Universal vaccines for polio, measles, smallpox, and many other diseases offer protection for years or even a lifetime.

But it is extraordinarily difficult. Universal vaccines work by prompting the immune system to manufacture antibodies aimed at relatively fixed targets—the so-called conserved parts of a virus that rarely mutate. In the coronavirus, however, the conserved parts are largely sheltered inside the stems of its infamous spikes, while current vaccines aim at the spikes' flashy tips. And although the tips are the most accessible parts of the virus, they are also the most frequently mutating—forming a halo of constantly moving targets. It's a common problem; viruses like the ever-evolving influenza present vaccine makers with the same challenge. In fact, nearly a century of effort has failed to produce one universal flu shot.

Pamela J. Bjorkman isn't daunted. A structural biologist at Caltech—and longtime collaborator of Rockefeller immunologist Michel C. Nussenzweig—she has spent decades studying how the immune system recognizes invading pathogens, working to develop therapeutics that make it respond in novel ways.

Bjorkman envisions a near future of universal vaccines for HIV, influenza, and the coronavirus. Though her efforts with HIV and influenza haven't yet panned out, her work with SARS-CoV-2 may be about to hit the mark.

Recently, Bjorkman's team reported in *Science* that they've created a new kind of training wheels for the immune system: a mosaic of different SARS-like coronavirus fragments arranged on nanoparticles. In animal models, the mosaic successfully sparked the immune system to produce antibodies against conserved parts of the coronaviruses and exhibited protection against both the original SARS-1 virus and the COVID-causing strain.

We spoke with Bjorkman about how this new approach might goose the immune system into seeing viruses differently.

With numerous therapeutics being developed against COVID, why is a vaccine still key to ending the pandemic?

I'll give you a great example: Early in the pandemic, Michel Nussenzweig and his team isolated many different antibodies from infected people. Some were really potent. Companies then made drugs based on these types of antibodies, and they worked!

But of course, the virus mutated, and those antibodies don't work as well anymore. Some, in fact, don't work at all against newer SARS-CoV-2 variants like omicron, which has been able to evade even the most potent antibodies. We really need a pan-coronavirus vaccine that protects against everything that's now out there and whatever might come next. But since coronaviruses differ so much, that might be extremely difficult or even impossible. So instead, my team is focusing on a coronavirus subset called sarbecoviruses, which have caused most of the recent spillover events—specifically, the original SARS-1 and SARS-CoV-2. We think a pan-sarbecovirus vaccine might be achievable.

LANCE HAYASHIDA / CALTECH

How does your approach differ from that of current vaccines?
Most of the neutralizing antibodies we make against SARS-CoV-2, or in response to a vaccine, target the tip of the receptor binding domain (RBD) on the spike protein. That's the region of the spike showing the most variability across SARS-CoV-2 variants and many sarbecoviruses. The RBD also has conserved parts—regions where the virus does not change often or at all. But these regions can be hard for antibodies to access, which could be partly why the immune system doesn't naturally tend to make as many antibodies against them.

"Experts have been saying, 'Wake up, world. This will happen again.'"

Our idea was to make a new target by gathering RBDs of eight different sarbecoviruses. And because the immune system tends to produce antibodies against identical pieces that are near each other, we randomly arranged 60 of these RBDs on each nanoparticle so that any two adjacent RBDs are rarely from the same virus. In a random arrangement, it's the conserved parts of the RBDs, which are similar across sarbecoviruses, that are more likely to end up next to each other, making them more visible to the immune system. The goal is a vaccine that encourages the body to stop making so many antibodies against the variable RBD tip and instead make more against the conserved part of the RBD.

But you initially developed this approach for other diseases.
That's right—we've spent five years making mosaic nanoparticles for influenza and HIV vaccines, but they didn't work well in generating broadly cross-reactive antibody responses. To our immense surprise, the approach turned out to work quite well for sarbecoviruses, however. In animal models, we got the types of cross-reactive antibodies we wanted. And not only do they bind to and neutralize the different kinds of sarbecoviruses that were represented on the nanoparticles, these antibodies also target sarbecoviruses that weren't represented in the mosaic. These mismatched neutralizing responses are very encouraging, because they show us what could happen in the event of a new viral spillover from animals to humans or when new SARS-CoV-2 variants emerge. It's a strong suggestion that a future vaccine based on our approach wouldn't need updating.

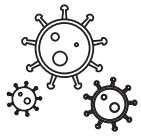
There's always the question, however, of whether the virus will still be able to mutate its way into escaping from these cross-reactive antibodies. I don't think that's likely because of structural limitations inherent to the virus itself. But we are testing that possibility in collaboration with Rockefeller virologists Theodora Hatziioannou and Paul Bieniasz. Using a SARS-CoV-2 spike protein displayed on an engineered virus that can safely be grown in the lab, we're looking for mutations that might occur after treatment with serums including antibodies derived from animals immunized by our mosaic.

You mentioned structural limitations in SARS-CoV-2. Are they the reason the mosaic strategy works well in this context?
The structural limitations are related to the position of the RBDs. The coronavirus's spike can enter a host cell only when one or more of its RBDs assume an "up" position; that's how it interacts with the cell's receptor. But the spikes can go into a "down" position as well—specifically, to help evade immune responses. When the RBD is down, the conserved part meets the rest of the spike protein. We think when we target this region with antibodies, the virus cannot mutate in response—it must remain stable in order to assume the down position. Yet it hadn't occurred to me when we started this work that this property of the RBD might be the whole key to why the mosaic approach seems to work for coronaviruses but not for other viruses.

Before COVID, some scientists kept research on coronaviruses alive even as public interest in them waned. Has the pandemic changed the calculus for how we support research into viruses that aren't currently causing trouble but one day might?
I hope so, but I'm not sure. Every 10 years or so, we have one of these spillovers—first it was SARS-1, then MERS, and now SARS-CoV-2. And if you look through the coronavirus literature, experts had been saying for some time, "Wake up, world. This will happen again."

But many researchers are already having trouble getting funding for coronaviruses. The same thing happened after the 2003 SARS-1 outbreak and after MERS as well. Once the storms passed, attention and support died down. Already with SARS-CoV-2, there are some who feel we're done with this. They might say, "We have vaccines, and we can keep updating them and move on." But I think that's shortsighted.

In the United States alone, hundreds of people are still dying from the disease each day, and long COVID can be devastating (read more about the condition in "When COVID lingers," on page 39). SARS-CoV-2 variants continue to emerge, and there's also the very real possibility that another animal coronavirus will spill over into humans; in fact, all that stands between us and a new pandemic is the emergence of a new virus that can infect humans and transmit asymptomatically. With respect to the current pandemic, it appears it could be making people more susceptible to other respiratory viruses, such as influenza and respiratory syncytial virus. So now we are seeing a worrying rise in cases for those viruses as well. I don't see this as being over yet. ◎



DATA

As of late last year, the WHO was monitoring 300+ omicron decendants.

Highly receptive

THE NEUROSCIENCE OF smell is supposed to be straightforward. Each olfactory neuron expresses a single olfactory receptor unique to a specific kind of scent. This streamlined system shows up across a variety of species, from flies to mice to humans.

But while studying *Aedes aegypti* mosquitoes, Leslie B. Vosshall and colleagues discovered that these insects aren't playing by the rules—and, as a result, have evolved a uniquely resilient sense of smell. This close-up of a mosquito antenna reveals an olfactory system that runs counter to conventional wisdom: Some odor neurons (shown in red and green) express one receptor, while others (yellow) are going rogue, displaying multiple receptors. "This was very surprising," says Vosshall, the *Robin Chemers Neustein Professor*, about her findings. "Mosquitoes unexpectedly pack a very large number of receptors into a single smell neuron."

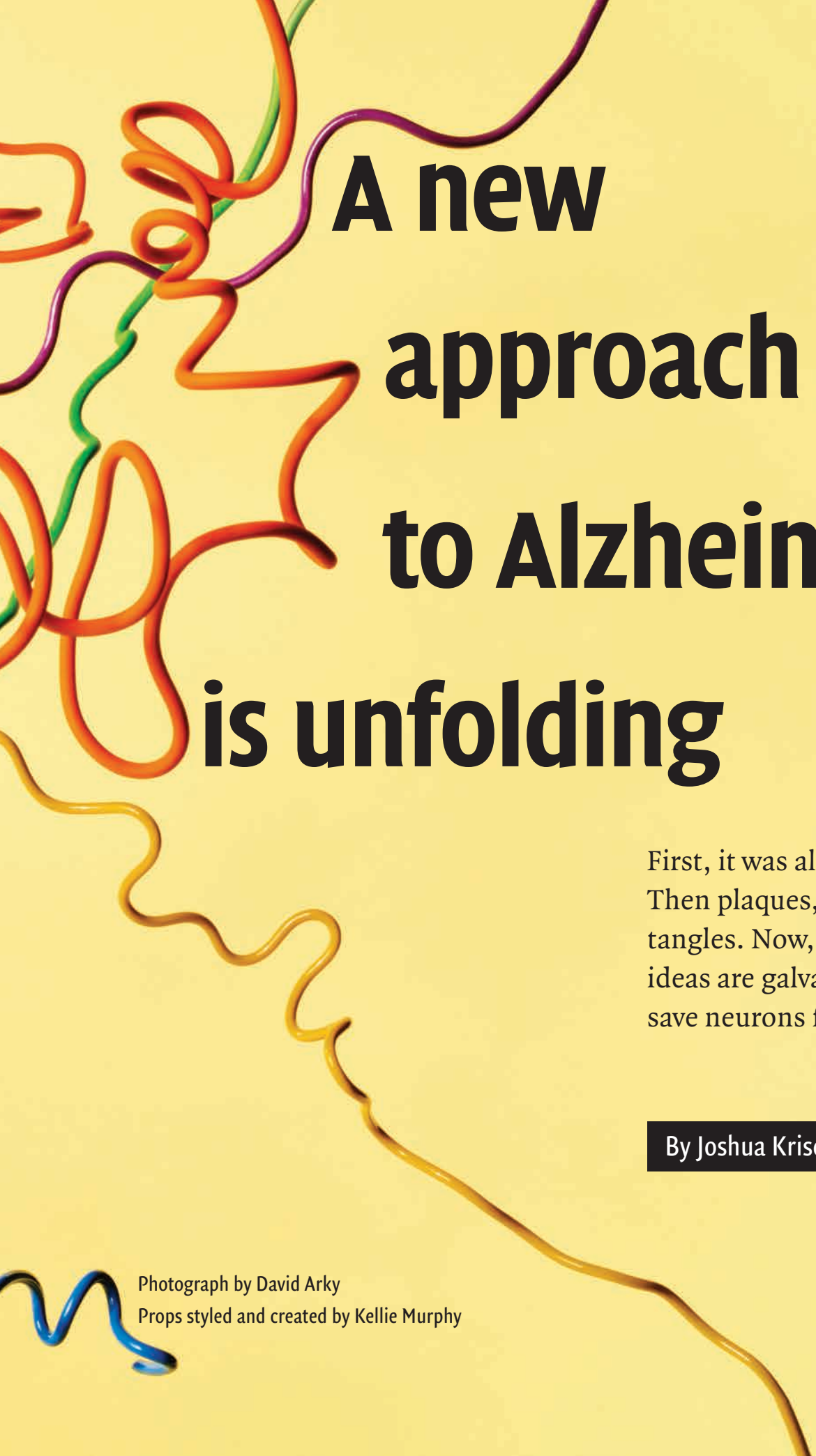
Vosshall notes that it's very hard to tamper with such a complex biological system, which may explain why she and others in the field have long struggled to come up with ways to manipulate a mosquito's sense of smell. Conceivably, a better understanding of how the system works could open the door to developing effective repellents or other tools to stop *Aedes* and other such pests from homing in on humans.

LABORATORY OF NEUROGENETICS AND BEHAVIOR

A new approach to Alzheimer's is unfolding

First, it was all about the plaques. Then plaques, and maybe tangles. Now, a plethora of new ideas are galvanizing efforts to save neurons from decay.

By Joshua Krisch



Photograph by David Arky
Props styled and created by Kellie Murphy

THE FIRST NEW Alzheimer's drug in 17 years should have been a blockbuster. But when the Food and Drug Administration approved aducanumab in the summer of 2021, it instead made headlines as yet another letdown in a decades-long quest for treatments against the disease. Because while clinical data showed that the drug did precisely what it was supposed to do—remove protein clumps from sick brains—it also failed where it counts most.

Patients were not getting any better.

"It was very disappointing," says Hermann Steller, *Strang Professor* at The Rockefeller University. "The field of Alzheimer's research has been focused on protein aggregates for decades; $30 billion has been poured into it. And there is very little to show for it."

Aducanumab was developed based on a hypothesis that has steered Alzheimer's research for decades. Alluring in its simplicity, this idea holds that neurons die mainly because the brain gets clogged by protein waste called beta-amyloid plaque and that clearing that waste, or preventing it from forming in the first place, will cure the disease.

But aducanumab was far from the first drug of its class to fail. Until recently, no treatment designed to rid patients' brains of plaques had turned out to meaningfully stop decline. And while the latest option on the horizon—a newer amyloid-clearing antibody called lecanemab—has shown some potential in late-stage trials, it also raises serious safety concerns. Even as it was unveiled, many scientists expressed only the most cautious optimism that treatments targeting plaques alone will be the answer to one of the most dreaded diseases on the planet. And for now, roughly 50 million patients around the globe continue to suffer, degenerating through memory loss, dramatic personality changes, and hallucinations, until those who live long enough to reach end-stage disease finally lose the ability to even speak or move.

Why has progress been so excruciatingly slow in the field of Alzheimer's, even after decades of scientific struggle?

For one thing, brain disorders in general—and neurodegenerative disorders like Alzheimer's and Huntington's in particular—present some of the most vexing challenges in all of medicine. By the time Alzheimer's symptoms manifest, for example, the brain is often too far gone to treat, and events driving the earliest stages of neurodegeneration have lain beyond scrutiny. Access alone presents a major obstacle: The strong bony helmet that protects our gray matter from trauma also makes it impossible to analyze brain tissue, and the barely permeable blood-brain barrier all but seals the brain off from the effects of would-be therapeutics.

MATTHEW SEPTIMUS

And the scientific challenges continue. "Mouse models that are so critical for research on other conditions often fail to capture the nuances of human neurocognition and how it deteriorates due to aging or disease," says Nathaniel Heintz, Rockefeller's *James and Marilyn Simons Professor* and director of the university's Zachary and Elizabeth M. Fisher Center for Research on Alzheimer's Disease. "What's left to look at is postmortem human brain tissue, which historically couldn't tell us much about what sparked neurodegeneration in the first place."

Research assistant Paul Darnell is using new molecular tools to characterize brain cells.

Nathaniel Heintz

Yet there are growing reasons to believe such historical challenges may become a thing of the past. Heintz and Steller, along with other scientists from various fields, are finding new ways to think about the disease, challenging long-held assumptions about amyloid and the very nature of neurodegeneration. This budding movement has all but discarded the notion of Alzheimer's stemming from a singular origin and replaced a once-dominating approach with a string of others. By focusing on how the brain interacts with multiple systems throughout the body and how the most overt organ decline may arise from the subtlest molecular disruption, these researchers are discovering multiple new avenues for treatment and developing new tools poised to take the study of neurodegeneration to the next level.

"**When research on** human neurodegeneration started decades ago, all they were able to see was the accumulation of plaques and tangles because that's what was visible using the technology of the day. So targeting beta-amyloid and tau made sense," says Heintz, referring to the main ingredients of protein clumps found in brains ravaged by Alzheimer's. "That was the most precise information available, and the amyloid hypothesis was generated from that data."

Trained as a molecular biologist, Heintz became interested in neurodegeneration about 10 years ago, bringing a new perspective to the study of brain disease. "Neurons in the brain function for decades and then suddenly succumb," he says. "Well, that looks to me like a biochemical problem in the cell." He recognized that without carefully examining that problem at the cellular and genetic level, scientists would keep stumbling in the dark in their efforts to prevent the cells from dying off.

But to even begin hunting for the root causes of neuronal decline, Heintz would need two things in exceedingly short supply: human data and, crucially, the ability to tell brain cells apart from one another. There are between 500 and 1,000 cell types in the brain, each with different molecular properties and gene expression patterns, making it extremely difficult to pinpoint which cells are malfunctioning in mixed samples. To sort out the good cells from the bad, Heintz teamed up with the late Nobel laureate Paul Greengard to develop a transformational methodology they dubbed translating ribosome affinity purification (TRAP) because of how it homes in on a cell's protein factory, the ribosome.

TRAP judges a cell's character by its actions, clocking every protein it produces as a proxy for its type. Heintz's group could now not only differentiate between the actors but also use their molecular signatures to identify who was disrupting the show—whether this immune responder, not that neuron, flubbed its lines—delivering an extraordinary tool for his own lab and others in the field that had been

spinning their wheels. The technique has already yielded tangible benefits. Heintz and Greengard, along with their Rockefeller colleague Jeffrey M. Friedman, initially used their new method to develop a cell-specific therapy addressing motor-related symptoms in Parkinson's disease and have since expanded on that work to study addiction, anxiety, and depression.

But Heintz sensed that Huntington's and Alzheimer's would be tougher nuts to crack. Because TRAP works best in animal models, his team went on to develop another tongue twister, fluorescent-activated nuclei sort sequencing (FANSseq). It works off a similar principle but with more subtlety, allowing scientists to characterize a cell by tracking the genes expressed in its nucleus. Only after applying FANSseq to the study of Huntington's—a comparatively straightforward disease caused by a known gene—did Heintz feel ready to set his sights on the thorny ambiguities of Alzheimer's.

Of all the unknowns surrounding this disease, the most confounding is how and when it starts. Scientists know it takes decades to develop, but no one has been able to pinpoint the precise genesis. Charting molecular changes over time would allow researchers to piece together the sequence of events that ultimately leads to full-blown disease, and this is where Heintz hoped his years of tracking biochemical nuance would give him an edge.

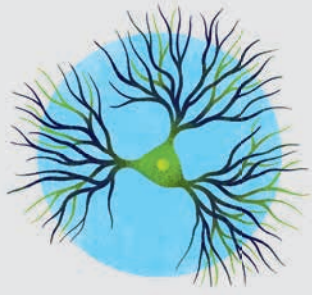
One way of looking for these early stages is to examine brain tissue donated by patients with Alzheimer's who die of other causes, like accidents or heart attacks, and then compare it with that of donors without Alzheimer's.

By using FANSseq to examine post-mortem tissue, the Heintz lab can study the state of each brain cell as it progresses through these diseases, generating findings that are helping researchers paint a fuller picture of how things begin to go wrong. In particular, studying why certain cell types are consistently the first to die off could point the way to slowing or stopping degeneration. There, Heintz has already spotted parallels with Huntington's,

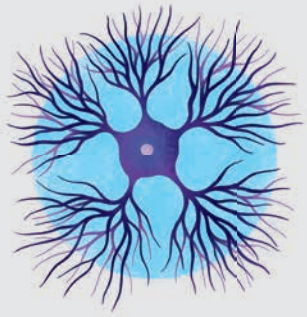
Scientists are focusing on how the most overt organ decline may arise from the subtlest molecular disruption.

Five fragile neurons

There are hundreds of neuronal cell types in the brain and hundreds of diseases in which one or several of these cell types fail—leading to problems with memory, mood, movement, and more. Here are some of the types most famously prone to decay.



Pyramidal neurons
Named for their shape, pyramidal neurons kick-start numerous cognitive and motor functions. Alzheimer's pathology disproportionately impacts these cells for reasons yet unknown.



Medium spiny neurons
These cells live mostly in the striatum, the brain's headquarters for motor and reward systems, and act as a circuit breaker to quiet neural activity as needed. They are the first cells to die in Huntington's disease, a condition caused by an inherited mutation.

Illustrations by Kasia Bogdanska

Heintz with graduate fellows Christina Pressl and Matthew T. Baffuto.

noting that the neurons that perish earliest are located in the cerebral cortex of Alzheimer's patients, right beside those most vulnerable in people with Huntington's. "It's a beautiful demonstration of how specific this pathophysiology is," says Heintz. "Our approach could soon provide additional insight into known mechanisms and reveal features that could offer new options for therapy."

AT LEAST ONE emerging pathway for treatment may come from a new twist on an old idea: While amyloid specifically may not prove to be the key cause of Alzheimer's disease, proteins in general could in fact be a culprit.

A cell's proteins live lives of constant flux—assembling, activating, and continuously adjusting until finally wearing out. At that point, each must be swiftly dismantled. If that process goes awry for any reason, toxic protein waste may accumulate throughout the body, potentially leading to muscle-wasting disorders or neurodegeneration.

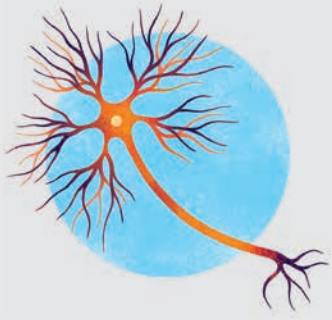
Steller was decades into his research on how the body breaks down excess proteins and why those systems sometimes fail when he was struck by the larger implications of a serendipitous discovery.

"It was an opportunity to make a contribution to the field of age-related neurodegeneration and offer a completely new explanation for how and why these diseases start," he says.

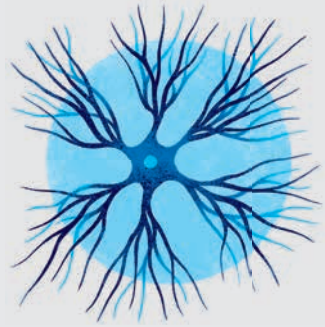
Steller had been studying proteasomes, the molecular machines that degrade proteins, when he discovered how these disassemblers travel long distances inside neurons. Proteasomes are made in the neuron's main body, but must do their jobs at the tips of its extensions, the long dangling threads that send and receive signals. If the neuron

MATTHEW SEPTIMUS

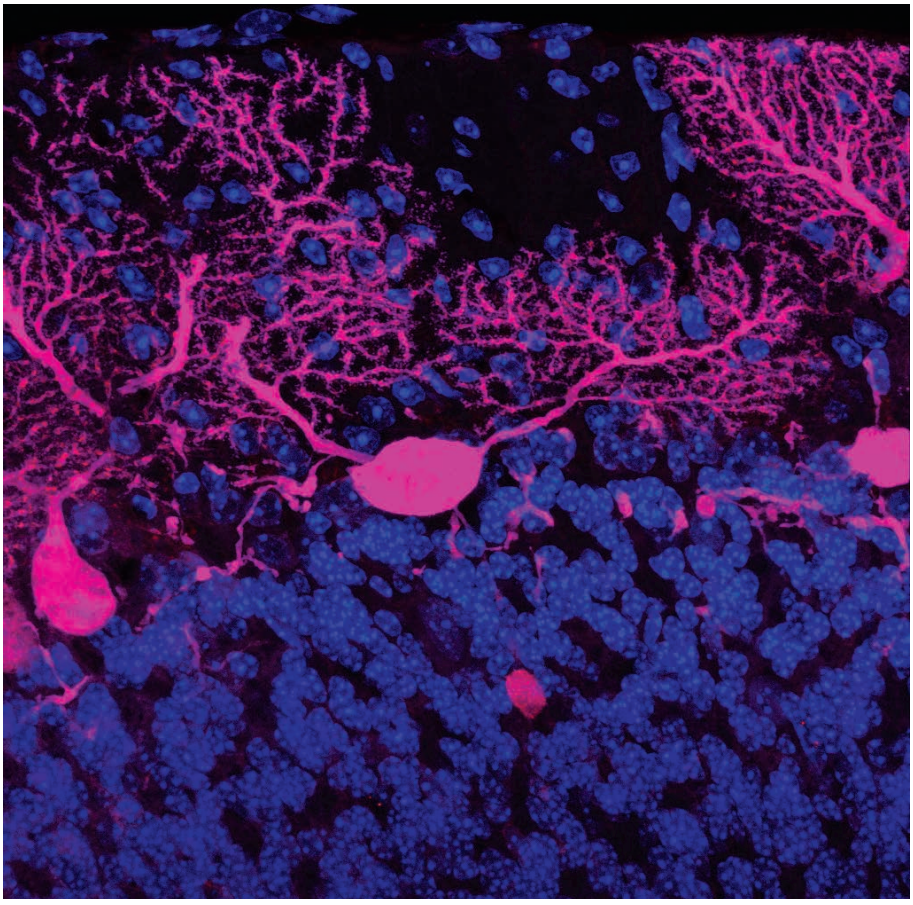
Purkinje neurons
These beautiful, bushy cells are found in the brain's cerebellum—a region responsible for movement, cognition, and emotion—as well as in the spinal cord. They deteriorate in a group of inherited neurodegenerative diseases, called spinocerebellar ataxia, leading to problems with movement coordination, among other things.



Motor neurons
These cells carry out voluntary movements like walking and talking, as well as involuntary ones like breathing. Their long neural extensions connect the brain and spinal cord with muscles throughout the body, some reaching all the way to the toes. When motor neurons degenerate in amyotrophic lateral sclerosis (ALS), muscles gradually weaken and ultimately atrophy.



Dopaminergic neurons
Named for their ability to produce dopamine, a neurotransmitter playing a decisive role in mood, stress, and addiction, these neurons also coordinate voluntary locomotion. Their loss contributes to the distinctive movement disorders of Parkinson's.



Research associate Junko Shimazu observes Purkinje neurons compromised by dysfunction of the protein removal system.

were the size of a basketball, Steller posits, its extensions would reach halfway across the width of Manhattan.

To get to where they need to be, proteasomes get loaded onto transporters—"like children on a school bus," Steller says—that ferry them across town. Recently, Steller's team reported that as the brain ages, the proteasome transporters become unable to keep up with increased demands for protein degradation. Over time, that translates into a progressive increase in protein damage as more stress gets added to the waste removal system. Eventually, there's a tipping point when there are no longer enough garbage collectors available to handle all the detritus piling up at the nerve endings. "We think that Alzheimer's, Parkinson's, ALS, and many other aging-related diseases may boil down to this system not working well enough as we age," Steller says. "It really is a weak link." When proteasomes fail to reach their destination, these accumulations can prevent neurons from communicating with one another, ultimately causing them to die.

There is ample support for Steller's hypothesis, including that people with a mutation known to stymie the activity of a proteasome regulator known as PI31 are predisposed to Parkinson's disease and that mutations in PI31 itself have been linked to Alzheimer's. And when the researchers jump-started the proteasome transport system in a mouse model of Parkinson's, this prevented neurodegeneration and greatly delayed onset of the disease.

Steller's lab recently devised a strategy to bolster that same pathway in human cells, with the ultimate goal to develop novel therapies for the treatment of neurodegenerative diseases. "We believe we've found a root cause of why we see declining function in neurons as we age," Steller says—a finding that has implications not only for neurodegenerative diseases but for the aging brain overall.

Hermann Steller

"Many aging-related diseases may boil down to the protein removal system not working well enough as we age."

"ISN'T IT STUNNING?" asks research associate professor Erin Norris, pointing to a bright crimson image depicting the mazy thicket of the cranial vascular system, which hangs on the wall of the Patricia and John Rosenwald Laboratory of Neurobiology and Genetics. Indeed, even with all the gray matter removed, it is easy to recognize the shape of a human brain just by the outline of the countless vessels that feed it. No other organ is so inextricably intertwined with our vascular system, nourished as it is by more than 400 miles of capillaries. And this makes the brain particularly vulnerable to vascular disorders, including those involving inflammation.

A highly choreographed process, inflammation is how the immune system cordons off an injury, rushes blood and other fluids to the site, and bombards it with scavengers seeking to destroy the threat, be it pathological (a nasty germ) or physical (a painful splinter). Once the invader has been neutralized, the process shuts down.

JOHN ABBOTT; JOSE RODRIGUEZ; CORBIS / GETTY

A first glimpse of the disease

Alois Alzheimer first laid eyes on a diseased brain in 1906, shortly after the death of one of his patients. Auguste Deter had been a poor homemaker suffering from memory problems, paranoia, and aggression. Alzheimer, a young psychiatrist with a burning interest in anatomy, placed a section of Deter's brain under his microscope. Gazing into the lens, he saw tissue clogged with thick deposits that would later be identified as amyloid plaques, and neurons notched with telltale whorls now known as neurofibrillary tangles.

Alzheimer believed he'd pinpointed the pathology behind a form of dementia, what he called "an unusual disease of the cerebral cortex." A few years later, the condition made its formal debut in a psychiatry textbook authored by his mentor, Germany's then-leading psychiatrist, who named it after Alzheimer.

Throughout the next century, researchers would chase the plaques and tangles that Alzheimer had painstakingly drawn in his case notes. In the 1980s, scientists identified the plaques as aggregates of a peptide known as beta-amyloid and the tangles as dysfunctional mutations of the protein tau.

Sidney Strickland

Sometimes, however, it gets triggered not by an external force but by an internal misfire, as with chronic inflammatory disorders such as lupus and Crohn's disease. Instead of healing the affected tissues, inflammation can then run amok, wreaking damage. Wherever it manifests, leaky vessels and dysfunctional clotting ensue.

Norris and her colleagues have long focused on clotting and inflammation, paying particular attention to Alzheimer's once they began to see striking findings emerge from a series of experiments designed to explore how coagulation and inflammation intersect with the disease.

Unexpectedly, this work revealed that knocking down a key component in the inflammation system rendered cells in the brain's hippocampal region resistant to decay. It was a pivotal moment. "We knew early on that the vascular system was involved in neurodegeneration," says Sidney Strickland, the *Zachary and Elizabeth M. Fisher Professor in Alzheimer's and Neurodegenerative Disease*, who heads the lab. "We just didn't know how."

The scientists began looking into familial Alzheimer's, a rare genetic version of the disease that strikes early and, tragically, is inescapable for those carrying the gene. "There are some people who we know, at birth, will invariably get Alzheimer's at around age 50, and there's no escape," Strickland says. Such patients offer scientists a unique window into the earliest stages of neurodegeneration—what the brain and body of a future Alzheimer's patient look like in childhood, adolescence, and early adulthood.

"Everything doesn't just collapse at age 50 with these patients," Strickland says. "It's been shown that things look abnormal decades before patients start experiencing cognitive impairment."

One glaring abnormality: high levels of deposited fibrin, the major protein component of blood clots in the brain. "We soon discovered it wasn't just higher levels of fibrinogen itself in early Alzheimer's," Norris says, "but also changes in inflammation, coagulation, and blood flow to the brain that seemed to come along with it."

"We aren't saying that amyloid plaques aren't involved in Alzheimer's," Strickland says. "We're saying that targeting plaques is not the only approach for treating this disease."

It makes sense that inflammation would promote neurodegeneration. "Neurons are fragile, delicate cells," says Marc Flajolet, a research associate professor in the lab formerly led by Greengard. With their exceptionally high energy requirements, neurons are frighteningly easy to kill and "are the first to die if there's a chronic problem," Flajolet says. One theory is that molecules secreted during chronic inflammation might be toxic to neurons; another line of reasoning goes that inflammation recruits molecules that eat up synapses, disconnecting neurons from one another and causing them to die.

Although these ideas were initially met with some resistance, researchers now generally embrace the importance of abnormal clotting and inflammation in Alzheimer's disease. Part of that acceptance came on the heels of another advance by Strickland's lab—the creation of a therapeutic antibody

"Everything doesn't just collapse at age 50. Things look abnormal decades before the onset of cognitive impairment."

Norris (center) with postdoctoral associates Ana Badimon and Daniel Torrente. Their group is developing an Alzheimer's drug that acts on the plasma clotting system.

JOHN ABBOTT

that mutes the plasma clotting system. When the scientists tested this drug in mice, they found that it significantly reduced the animals' inflammation and coagulation profiles. The team is now advancing their animal trials of the antibody with an eye toward moving it into the clinic.

IT REMAINS TO be seen whether the classic drug-development approach inspired by the amyloid hypothesis will on its own make a meaningful difference for patients. Whatever the case, important questions remain surrounding amyloid and its fellow traveler, tau. Flajolet's work, for instance, is predicated on the assumption that protein aggregates actually are the main problem—just not all aggregates of all sizes. "We believe tau protein aggregation, specifically, may be central to where and when Alzheimer's disease starts and to how it evolves and progresses over time," he says.

Until recently, tau played second fiddle to amyloid at least partly because there was no easy way to study its deposits in the brain, known as tau tangles, which are quietly ensconced within damaged brain cells (amyloid plaques, by contrast, tend to overtly gum up the cells' exterior). But then the FDA approved a new intravenous drug in 2020 that allowed physicians to track tau aggregates in a patient's brain and to image tau pathology via PET scan. Researchers now believe that the largest deposits—the infamous neurofibrillary tangles—are not where most of the danger lies.

"We believe the toxicity is not so much coming from fully formed neurofibrillary tangles as from their precursors, the smaller oligomers," Flajolet says. His team is currently looking for molecules that might block the formation of small tau oligomers, slow their aggregation, interfere with their spreading, or at least make them easier to track and study in the future.

Truth be told, even those scientists shifting away from amyloid and tau remain interested in the storied plaques that kicked off the study of neurodegeneration and all but defined the field until recently. "What we're finding isn't running counter to conventional wisdom so much as adding nuance and value and insight," Heintz says.

The widening scope of Alzheimer's research has yet to bear fruit in the form of novel therapies. But with new perspectives and tools in hand, we are in a better position than ever to get to the roots of a problem that has claimed memories, personalities, and lives for millennia—and, ultimately, to understand how that problem might be fixed.

"Maybe it'll be neuroinflammation or a new take on tau or something we haven't even considered yet," Heintz says. "But I have no doubt we're going to see new and effective therapies unfold in the decade ahead." ◎

What you get is not just what you see

Scientists have built a novel AI system that rewrites the rules for computer vision.

It might soon turn neuroscience on its head.

By Bahar Gholipour

The picture is unmistakable: a pepper sliced in half. Yet when Winrich Freiwald projects it on the big screen during a recent lecture, soft giggles erupt from the audience.

Because while all that's there is one half of a vegetable, it's nigh impossible not to see something else—a spooky green face, with holes for eyes and seeds for teeth, staring anxiously ahead. "We know full well it's a pepper," Freiwald says, his long legs pacing the stage of Rockefeller's Caspary Auditorium. "But we cannot help seeing the face."

Illustration by Saiman Chow

If the brain is simply processing incoming cues, how does it quickly turn ambiguous data into coherent representations of objects and scenes?

It's not our fault; our brains come equipped with a neural machinery whose sole task is to perceive and recognize faces. This internal face detector never rests—every time certain complex patterns hit the retina, it gets activated (see "Why there's a man in the moon," on page 31). To a neuroscientist, the phenomenon is not just comical but consequential. Pepper faces, along with a host of similar illusions, illustrate profound mysteries about the brain and its relationship to the world around us. Vision may be the best understood of the brain's functions, yet we seem to have misunderstood something about the way our minds derive meaning from visual inputs.

"Examples like this suggest that when we see something, the brain is doing a lot more than just registering light," Freiwald says, referring to the textbook description of how we see: Light bounces off an object, hits the retina, zooms along the optic nerve, and, voila—electric signals are transformed by the brain into a teacup. For one thing, this canonical understanding of the visual system doesn't account for the fact that a pepper seed isn't always a pepper seed but can register as a tooth under certain circumstances. And if the brain is simply processing incoming cues, how does it quickly turn ambiguous data into coherent representations of objects and scenes, like when you recognize your grandmother's cheerful face in a blurry, old photograph?

Freiwald is among a growing circle of scientists turning to a radically different view, one that posits that what we see isn't merely a reflection of what's out there. It is more akin to a mental construct, something cognitive scientists call inference. "We think the brain has some kind of internal component that not only detects incoming stimuli but also generates them," he explains. "In a manner of speaking, the brain is constantly hallucinating."

A few years ago, Freiwald teamed up with computational cognitive scientists Joshua B. Tenenbaum and Ilker Yildirim, who concocted an idea for a system to test this generative theory of vision. Together, the scientists set out to build a new kind of artificial intelligence to explore whether the process by which we recognize faces or other objects starts in the brain itself. Among the things they wanted to know was whether a machine could be programmed to match observations made in biological experiments. If it could, there would be far-reaching implications for neuroscience. And it gradually became clear that their work might have ripple effects: Machines that don't just think faster than us but also, on a cognitive level, behave more like us could help propel advances in everything from developing safer autonomous vehicles to slowing climate change.

But much would depend on what the scientists learned.

13

Number of milliseconds it takes for the human brain to see an image.

Faces are an elite category of human perception. They are among the first things we learn to look at as infants, and as we grow older, our social functioning relies heavily on the ability to recognize family members, friends, and foes and read the facial expressions of people we interact with. This may be why humans and other primates have evolved specialized brain cells just to recognize faces. "It's a notably inefficient use of neurons," Freiwald says. So much so that when he first heard about this phenomenon during his graduate studies, he rejected the notion. "I thought, that's not an elegant solution for the brain," he says. "To have neurons that respond only to one object category and not others? That is odd."

Even Charles G. Gross, a cognitive neuroscientist at Princeton University who first discovered face neurons in the 1970s, was baffled. It took another two decades before MIT neuroscientist Nancy Kanwisher identified the fusiform face area, a region in the brain's inferior temporal cortex that is specialized for face recognition. Freiwald trained in Kanwisher's lab as a postdoc, then joined Margaret Livingstone at Harvard Medical School, where he worked with then colleague Doris Tsao to combine brain imaging studies with recordings of individual neurons. The scientists ultimately uncovered a network of six pea-sized patches composed almost entirely of face neurons.

Since then, Freiwald has been able to characterize these patches in great detail. Among his lab's findings is that each patch processes a different dimension of facial information. In one of the first patches to get activated, for example, neurons are sensitive to facial features, such as the distance between a person's eyes. In one of the middle patches, neurons code for orientation—some favor right-side profiles; others half profile. And neurons in the last patch respond to faces as a whole, no matter their orientation.

Having deciphered the functions of the face patches, Freiwald was able to chart the itinerary of a face as it travels through the mind, transforming from visual input into recognized object. And along the way, he saw things he wasn't able to explain.

In one set of experiments, Freiwald's team showed macaque monkeys renderings of human faces seen from various angles while monitoring neuronal activity within the face patches. In one of the middle patches, neurons responded differently to pictures of the same face seen at different angles, as the scientists had expected. But there was one bizarre exception: When the monkeys saw mirror-reflected poses—say, one picture of a face turned 45 degrees left from center and another with the same face turned 45 degrees in the opposite direction—the neurons responded as if the two pictures had been identical.

This mirror-symmetry effect was a mystery. In real life, faces don't suddenly jump from left to right; they rotate from one pose to the other. Freiwald and his colleagues couldn't explain, at least not within the conventional framework for how vision works, why the neurons were programmed for mirror symmetry. Had we gotten something fundamentally wrong about how the brain is wired?

"What I cannot create, I do not understand," the theoretical physicist Richard P. Feynman famously said. And for cognitive

Why there's a man in the moon

Humans are so good at spotting faces in inanimate objects that psychologists have a word for it: pareidolia. The phenomenon isn't actually confined to faces; people are fully capable of finding meaningful images in any random visual pattern (just ask Hermann Rorschach, the early 20th-century psychoanalyst) or even human speech in garbled auditory stimuli (just ask Paul McCartney). Research has shown that those who believe strongly in a higher power or the supernatural are more likely to see a visage in their toast.

KIK SAM; VALERIANA Y; ANNFOSSA / SHUTTERSTOCK

Freiwald has characterized the primate face perception system in great detail.

MATTHEW SEPTIMUS

neuroscientists, one way to understand how the brain operates is to create AI systems that emulate its computational principles.

An auspicious encounter presented Freiwald with the opportunity to do just that. In 2013, he arrived at the newly launched Center for Brains, Minds, and Machines, a multi-institutional forum located at MIT that brings together scientists working on biological and artificial intelligence. It was there that he first met and began collaborating with Tenenbaum, a computational cognitive scientist at MIT whose work focuses on understanding how the brain makes inferences from sensory data, and Yildirim, a postdoctoral researcher co-mentored by Freiwald and Tenenbaum now on the faculty at Yale University.

Together, the three scientists began imagining a new kind of AI that could be trained to recognize faces. A cousin to the system that unlocks smartphones, theirs would be able to make inferences and thus generate new data in addition to processing incoming pixels. If successful, it would provide an experimental system for studying some of the most elusive aspects of being human, like how we effortlessly arrive at our commonsense understanding of the world, so rich in detail and meaning, when all we have to go on are visual cues that often contain a bare minimum of information.

Or, as Tenenbaum once put it: "How do humans get so much from so little?"

AI IS CREEPING into our lives. It proofreads our emails, curates our social media feeds, and checks our credit cards for fraudulent activity. Yet that is nothing compared with what the technology promises to do in the future: write newspaper articles, tutor students, diagnose diseases.

In fact, there are already computer-vision machines that outperform doctors in detecting and classifying skin cancer. Like many other tech marvels—Siri, chatbots, Google Translate—they rely on deep neural networks, or deep nets, AI systems designed to operate like the networks of neurons in the human brain. Generally, the deep nets used in computer vision reflect the conventional understanding of human vision, consisting of an input layer and an output layer with more interconnected layers in between. Like human toddlers, these systems can be trained to recognize objects by essentially being told what they're looking at, and they continuously recalibrate internal connections until they're able to correctly associate patterns in the data with the right answer.

Once trained, a deep net can be unleashed to classify an input it hasn't seen before. If it sees a sycamore tree for the first time, for example, it may have seen enough other tree species to correctly

What is common sense, scientifically speaking? What is that crucial thing that most of us can relate to yet struggle to define?

identify the sycamore as a tree. An untrained system, on the other hand, might classify the sycamore as broccoli.

But even the most advanced deep net behaves differently than the brain in several important ways. For example, it may require thousands of hours of training with millions of examples before it can accurately distinguish trees from broccoli, while a human toddler easily learns to categorize these objects after seeing just a few examples. Moreover, even after extensive training the system will occasionally make errors that no human would make, like mistaking a squirrel for a sea lion. And just a little bit of visual noise, which humans will easily ignore or not even notice, can break down the AI entirely. (To be fair, humans make mistakes, too, but ours are very different in nature. We fall for optical illusions that AI is completely insensitive to, like when we lose our way trying to trace the path of an impossible staircase in an M.C. Escher print, for example.)

"Clearly, our conventional deep nets are missing something crucial," Freiwald says. "They seem to lack common sense."

But what is common sense, scientifically speaking? What is that crucial thing that most of us can relate to yet struggle to define? Important clues may lie in the generative mechanism that Freiwald and others find in the seeing brain: It isn't merely recording what's "out there," as most computer-vision systems are trained to do, but also dreaming or hallucinating (in a healthy way) to proactively produce our perception of the world.

1

Smallest number of photons a human eye can detect, according to a 2017 Rockefeller study.

The concept of vision as a generative process best surfaces when it produces the wrong answer. Think of a time when you momentarily misperceived an object for another—maybe a hose on the ground made you think you were about to step on a snake, for example. For a split second, your brain conjured a distinctive image of the snake—until you realized your mistake, at which point the slithery creature instantaneously transformed into a harmless piece of plastic tubing. But where did that snake come from in the first place?

The 19th-century polymath Hermann von Helmholtz was among the first to point out the neuroscientific significance of such quotidian delusions. He recognized that when we see an object, the light that hits the retina is often less than ideal—the room might be dim, the object might be partially hidden from view, or a cacophony of other visual stimuli might be distracting us. How does the brain, almost always, correctly see through all that ambiguity? Helmholtz suggested that vision is a kind of inference in which the brain produces a hypothetical object—in much the same way it churns out images when we are dreaming—and then uses the actual sensory input to confirm its hypothesis.

According to this theory, the brain is much more creative than we've been giving it credit for. One step ahead at every turn, it doesn't just process incoming information but also tries to deduce the causes behind it. When we see an object, the brain offers up its best guess about what we're looking at (could that thing on the table be a teacup?). Then it collects incoming data to fact-check that inference (indeed, it is a bowl with a handle, just like other teacups I've seen before). What we actually perceive, then, is a dreamlike simulation of the object, originally produced by the brain and subsequently refined by sampling data received with the eyes.

Many of Helmholtz's contemporaries dismissed this idea, however. And although his inference theory gained some popularity among cognitive scientists in the late 20th century, it never quite took off, partly because scientists couldn't reconcile an elaborate inference process with the breakneck speed of biological vision.

It wasn't for lack of trying. Yildirim points to recent efforts to build a generative computer-vision system based on the inference approach. No matter how those systems were engineered, they required extensive iterative processing that took much longer than the 100–200 milliseconds the brain takes to perceive a detailed scene. "For both AI folks and neuroscientists, it has been unsettling that the

process would be so cumbersome and slow," he says. "No one believed that this could be the way the brain works, because our perception is nearly instantaneous."

The team had an idea for how to create a supercharged, generative AI system. They essentially combined the best features of two approaches—the speed and processing power of established deep nets and the inference ability of a generative system—to build a new computer-vision machine dubbed the efficient inverse graphics network, or EIG. The goal was to use it as a model for the brain's face-perception machinery, "arguably the best studied domain of high-level vision," Yildirim says.

While deep nets are conventionally trained to classify objects, starting from pixels and working up, Yildirim and his colleagues put the EIG through a different educational program. They showed the machine 3D renderings of 200 human heads and taught it to detect the underlying image structure, breaking down the images into their basic components. The system's deep net was able to do this because Yildirim had equipped it with an inverted version of a graphics processing unit, or GPU, a computer chip that enables the quick rendering of 3D graphics and animations for computer games, among other things. But instead of creating graphics, the deep net ran its GPU backward to deconstruct the renderings that it was shown.

To see whether the EIG would offer a more realistic model of human perception, all that remained was to test it.

IT WORKED. WHEN Yildirim showed the EIG a 2D image of a face it hadn't encountered during training, the machine moved through its programming to arrive almost instantaneously at a 3D version of that same face. More important, its inside layers evidenced the same properties that Freiwald had spotted in the brain, including the mirror-symmetry effect. "The EIG mimicked the main stages of face processing and reproduced the physiology," Freiwald says.

To the scientists' delight, their AI system also mirrored human vision at the behavioral level. For example, it made humanlike mistakes when the researchers tested it on visual illusions like the hollow mask (see an example at https://go.rockefeller.edu/hollowmask). In the classic rendering of this illusion, a face appears to be rotating from right to left. When it reaches a half rotation, you realize you have been looking at the back of a mask—a concave rendering of a face. Yet as the face keeps rotating, the illusion resurfaces again and again; even though you know you're looking at a hollow mask, you'll continually revert to seeing a regular, convex face, as if your brain insists on interpreting the incoming information in the context of ordinary faces.

> The brain may be much more creative than we've been giving it credit for. It doesn't just process incoming information but also tries to deduce the causes behind it.

Whereas conventional AI doesn't fall for the illusion, the EIG misperceived the concave mask in the same way we do. And a look at the machine's inner workings confirmed that it had computed the incoming light erroneously. "It's one line of evidence that the human brain is really doing the inference approach, very different from that of a conventional deep net," Freiwald says.

2nd

Rank of the human eye in terms of the complexity of organs in the body. Only the brain tops it.

All of which suggests something truly remarkable about the brain, according to the researchers: When we see a face, a teacup, or anything else, our brain infuses the object with an interpretation, producing a richer bounty of data than the object itself provides. This inference might explain how we can learn so much about what we're looking at so quickly, potentially providing a recipe for a crucial aspect of human intelligence, perhaps that common sense that AI systems famously lack.

"When you see a picture of Audrey Hepburn, you don't just see a two-dimensional arrangement, you're inferring how the face looks in 3D," Freiwald says. "Yet that information is not really there in the picture itself. That we get more out of an image is a form of intelligence."

The source of this intelligence, or what makes such inferences possible, can be thought of as knowledge structures embedded in our brains, that guide our perception, thinking, and actions. Such

Yildirim combined the best features of deep nets and GPUs to build the team's new AI.

MATTHEW SEPTIMUS

knowledge has likely formed partly during evolution and partly through early life experience, as when infants develop an understanding of gravity by dropping their sippy cup. Once we've figured out this basic law of physics, that knowledge stays with us and is called on each time we catch a falling dish.

"Our thinking is structured around a basic understanding of the world in terms of physical objects and entities, other humans and animals, and how they interact," Tenenbaum recently said at a conference at Cold Spring Harbor Laboratory. Such phenomena are sometimes referred to as "intuitive theories." Tenenbaum calls them our commonsense core.

"**The EIG is** taking us a step closer toward reverse-engineering the human brain," says Yildirim, who is currently teaching the machine to move beyond faces—recognizing whole bodies, places, and even how physical objects move and react to external forces. "That means we're also on a good course to ultimately advance the potential of AI."

Computers are brilliant except when they are stupid. Notwithstanding the stunning advances in AI in recent years, progress in the field is now facing an impasse. Self-driving cars won't be zooming down the roads anytime soon—not as long as they abruptly slam on the brakes when tricked by soap bubbles. And no home helper robot can come online until we can trust it won't load the cat into the dishwasher. The future will tell whether these systems can become wiser by incorporating generative-processing capabilities like those of the EIG.

Yet to Freiwald, just as exciting is what the EIG and similar systems might do for neuroscience. "Building a machine that recognizes faces the same way a primate brain does is a huge milestone," he says. "It shows us that we've correctly understood this aspect of the brain's function and that we'll be able to apply that knowledge to study the brain's functions more generally."

Because how we see a face tells us much about how we see the world, literally and figuratively. If perception is shaped by the brain, then it is fundamentally a cognitive act. And then the phenonenon of face processing, fascinating unto itself, becomes an entry point for exploring how neural processes translate into human nature: how the brain generates our thoughts, emotions, and behaviors and how we perceive others and adapt to a social environment. Moreover, this novel way of looking at the brain—as the active builder of our models of the world—provides new frameworks for studying the mechanisms behind autism spectrum disorders and mental illnesses such as bipolar disorder and schizophrenia.

"It's still extremely early days," Tenenbaum noted during the conference. "And that's the most exciting time." ◎

Maybe the virus isn't the problem

By Eva Kiesler

Three years into the pandemic, the scientific response has delivered beyond expectations. We have effective vaccines against COVID, we can treat it with antivirals, we have practical strategies for avoiding it. Yet how the virus impacts people's health can still seem mysterious. Why is it, for example, that some end up on ventilators and others get by with a scratchy throat or no symptoms at all? Why do some recover fully within weeks while others struggle for months or years with long-COVID symptoms? And most puzzling of all, why do some individuals appear to have dodged the virus this whole time?

Answers are now emerging from hundreds of scientists around the globe who joined forces when the first wave got underway. As the world fixated on the new virus, these experts looked beyond that spiky orb to focus on the human side of the equation—specifically, on variations in our genome that determine what will happen when a person comes in contact with SARS-CoV-2. Their discoveries are changing the way we think about infection, not only in the context of COVID, but for any illness set in motion by a microbe.

Illustrations by Gracia Lam

In the winter of 2020, right after news emerged about a new coronavirus outbreak in Wuhan, China, Jean-Laurent Casanova, an immunologist with laboratories in New York and Paris, sat down to write the most important email of his career. He would send it to nearly a thousand people. "I wrote everyone I had ever worked or corresponded with," he says. "Absolutely everyone."

He is an avid correspondent, with friends and collaborators around the world. But this email was different and would quickly move the needle on research into COVID.

"There's been a stunning inter-individual variability between patients," he typed, referring to the erratic way in which the disease was rippling through communities. It was already evident that elderly people and those with underlying conditions were at risk for the worst outcomes; but something strange was happening among those who were young and previously healthy. While most had manageable symptoms or no symptoms at all, a seemingly arbitrary group was contracting severe or fatal forms of the disease.

There was no way to predict who might live and who might die from an infection, and the uncertainty was setting off a secondary pandemic of anxiety and fear. But Casanova, the *Levy Family Professor* at Rockefeller, had a strong suspicion where the reasons for the discrepancies might lie.

"Please join our effort!" he continued in bold, red font. "We need whole blood, fresh or frozen, or genomic DNA from patients."

Hundreds of his colleagues responded, and thus was born the COVID Human Genetic Effort (CHGE), an international consortium that Casanova co-leads with Helen Su, chief of the Human Immunological Diseases Section of the National Institute

"We have extensive knowledge about pathogens, but we still don't understand why they make some people critically sick."

of Allergy and Infectious Diseases. Now in its third year, CHGE brings together more than 400 scientists and close to 40 DNA sequencing labs across six continents, and it's well on its way to answering the pandemic's most perplexing questions.

In sequencing and analyzing patients' genomes, the consortium has already found flaws in immunity that might explain up to 20 percent of critical cases, an achievement stemming from discoveries made in just a handful of people. It's the kind of work that simply cannot be done without a global reach.

"If you're looking for a rare mutation, there may just be one person in Hong Kong and another person in Buenos Aires who have it," Casanova explains. "And you will never find it unless you have colleagues who can help you recruit patients from these places."

He would know. For over 30 years, Casanova has leveraged an extensive professional network to investigate why people may respond differently to the same pathogen. Ten years ago, for example, his team found one patient in Turkey and another in Iran who carried a rare mutation that made them abnormally sick from generally harmless mycobacteria. Another time, they studied a French 2-year-old with a different mutation who nearly succumbed to the seasonal flu, which most children fight off within a week.

So when COVID hit, Casanova and his colleagues immediately got to work collecting DNA from as many infected people as they could get their hands on. While others focused exclusively on the new coronavirus, these scientists believed the biggest mystery of the disease lay elsewhere: in the tangle of biochemical pathways that make up the human defense against it and other pathogens.

"What we're seeing in this pandemic was also true for the 1918 flu and almost any other infectious disease," says Isabelle Meyts, a professor in pediatrics at KU Leuven in Belgium and member of CHGE's steering committee. "Only a fraction of infected people gets life-threatening disease. And the problem isn't the pathogen, it is us—the problem is the genetics of the host."

485

Number of diseases linked to inborn mutations in immune-system genes.

IN THIS SENSE, infectious diseases are like cancer, cardiovascular disease, diabetes, and almost every other illness that scientists have scrutinized at the molecular level. They can be traced to mutations in the genes we are born with, and when such mutations are discovered, new opportunities may arise to diagnose, treat, or prevent the condition.

Thus far, however, infectious disease experts have often ignored the role of human genetics, focusing instead on the study of microbes—how they spread, how they enter host cells, how they replicate, and how to kill them. The omission dates back to the early pioneers of the field, who were more interested in germs than in genes.

This was true even for Louis Pasteur, the 19th-century chemist who discovered that microorganisms spread disease and turn grapes into wine. He was among the first to point out that infectious diseases are both infectious and inherited. Of flacherie, a silkworm plague that nearly put an end to the French Second Empire's silk industry, Pasteur wrote that "it is not the microbe that is transmitted from parents to offspring, but the predisposition to disease." Still he didn't dwell long on the Mendelian aspects of infection, turning instead to more pressing matters—like convincing the world that common diseases like rabies and typhoid fever were caused by microbes rather than by "bad air" or bad morals.

As the genetic aspect fell by the wayside, Pasteur's and other germ theorists' work on pathogens led to spectacular new therapeutics, including vaccines and antibiotics that revolutionized public health. Yet strangely enough, these scientists had little or no

knowledge of how their innovations worked or how infectious pathogens were sabotaging their hosts' physiology in the first place.

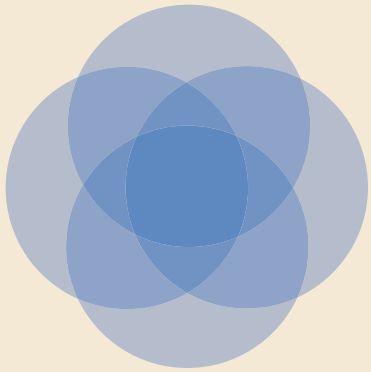
"Medicine usually follows science," Casanova says. "But for infectious diseases, the opposite happened: Medicine got ahead of scientific understanding very early on, and the science still hasn't caught up. We have extensive knowledge about pathogens, but we still don't understand why they make some people critically sick."

PICKING UP WHERE Pasteur and his contemporaries left off, Casanova and Meyts are now shifting the focus from pathogens back to their hosts—which is to say, to the patients themselves. Both are pediatricians interested in inborn errors of immunity, mutations that some people carry from birth that compromise the body's ability to defend itself against specific pathogens (or in some cases, against multiple pathogens). Inborn errors represent the most clear-cut cases of genetic predisposition to infectious disease, and as such, they tend to be rare. Most of them are subtle as well: If you're carrying an inborn error, you may not know it until you're confronted with a germ capable of exploiting that particular flaw.

In CHGE's first initiative, Casanova, Meyts, and their colleagues recruited hundreds of patients with critical COVID—those who were ill enough to be admitted to an ICU—as well as individuals with a mild or asymptomatic infection. They then compared genomes, looking for telltale differences between the two groups. Just a couple of months into the pandemic, results emerged that were so illuminating that "COVID may already be the best understood of all infectious diseases," Casanova says.

The researchers found that at least one to five percent of the critically ill carried inborn errors in gene coding for molecules that control the activity of type I interferons, a set of signaling substances released by our cells in response to some viral infections in order to prevent those viruses from replicating. Because of their warped interferon activity, these patients' immunity lacked the ability to curtail the virus in the early stages of the disease, a glitch that may allow SARS-CoV-2 to move from the respiratory tract to the bronchi and lungs and ultimately to reach vital organs throughout the body (see "When interferon fails to interfere," on page 40).

When COVID lingers



JUST A FEW MONTHS into the pandemic, something quite unexpected happened: Swaths of previously healthy people began to report COVID symptoms lasting long after the acute phase of infection.

At Karolinska Institutet in Stockholm, Petter Brodin, an immunologist and specialist physician, is leading the COVID Human Genetic Effort's research into long COVID. He and his co-workers have recruited patients whose blood samples might provide clues about the way in which SARS-CoV-2 and many other pathogens wreak havoc in the body for months or years. Their work is complicated by the fact that long COVID may not be one syndrome, but rather an umbrella encompassing at least three different scenarios. "In some patients, the disease may be driven by autoimmune responses," Brodin explains. "In others, it may occur because the virus triggers an overblown immune response in which large numbers of T cells are activated."

In addition, Brodin is seeing growing evidence for a third scenario: that small factions of live virus can persist in some organs, including the lungs and GI tract, where they keep on replicating indefinitely. He would like to know if viral hideouts also play a role in post-infectious conditions that may develop after influenza, mononucleosis, or Ebola, or in a poorly understood condition called myalgic encephalomyelitis or chronic fatigue syndrome. All of these diseases have features in common—you might think of them as overlapping circles in a Venn diagram.

A similar relationship exists between multisystem inflammatory syndrome in children (MIS-C), a dangerous illness that some kids develop after SARS-CoV-2 infection, and another pediatric condition known as Kawasaki syndrome. In both diseases, vital organs like the heart become critically inflamed. Shen-Ying Zhang, an associate professor of clinical investigation in Casanova's lab, says that around 30 percent of children with MIS-C are diagnosed with Kawasaki syndrome when they first show up in the clinic.

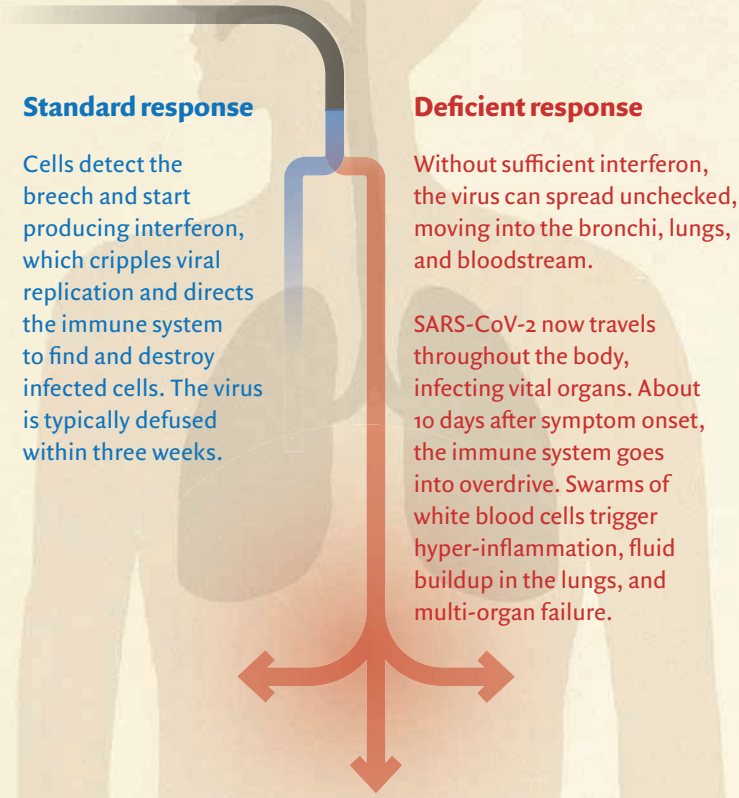
Recently, Zhang and her colleagues linked MIS-C to mutations in three genes that are part of the same immunological pathway. "So far we've detected these inborn errors in five patients," she says, "three of whom had SARS-CoV-2 Kawasaki-like disease."

The scientists believe that these errors thwart the immune system's ability to cope with not just SARS-CoV-2 but also several other coronaviruses, including SARS-CoV-1 and others known to cause the common cold. They see Kawasaki syndrome as a catchall for all these post-viral illnesses, while MIS-C is specific for SARS-CoV-2.

"Our findings will pave the way for studies of classic Kawasaki disease and other childhood autoimmune diseases, as well as inflammatory post-infectious conditions," Zhang says.

When interferon fails to interfere

When SARS-CoV-2 infects a host, the virus enters the respiratory system and attacks vulnerable cells. Just days later, it's already replicating profusely. What happens next depends on the host's genetics—specifically as it pertains to the production and function of type I interferon protein.



Standard response

Cells detect the breech and start producing interferon, which cripples viral replication and directs the immune system to find and destroy infected cells. The virus is typically defused within three weeks.

Deficient response

Without sufficient interferon, the virus can spread unchecked, moving into the bronchi, lungs, and bloodstream.

SARS-CoV-2 now travels throughout the body, infecting vital organs. About 10 days after symptom onset, the immune system goes into overdrive. Swarms of white blood cells trigger hyper-inflammation, fluid buildup in the lungs, and multi-organ failure.

What's more, inborn errors turned out to be the tip of the iceberg. The team found that some critically ill people whose interferon genes were intact instead produced misguided immune molecules known as autoantibodies. In these patients, autoantibodies treat the body's own interferon proteins as foreign entities, glomming onto them and preventing the proteins from functioning properly.

Meyts was intrigued to learn that poor COVID outcomes may arise via two different mechanisms that both sever interferon signaling. "Whether the problem is autoantibodies or interferons or the genes that control interferons themselves, we see the same phenotype," she says. "These are beautiful findings that make a lot of sense."

Interferon autoantibodies are much more common than interferon mutations: In analyzing pre-pandemic blood samples from more than 35,000 individuals, the researchers detected them in about one in 500 people aged 18 to 69. The team also discovered that the chance of having autoantibodies increases with age—their incidence is 1.1 percent among people aged 70 to 79, for example, and 3.4 percent among those 80 and older—which may be why many elderly people die from COVID.

Taken together, the inborn errors and autoantibodies discovered thus far account for approximately one in five severe cases. But the researchers believe interferon deficit may be driving even more ICU admissions and deaths through mechanisms yet to be discovered. Moreover, the team recently detected interferon autoantibodies in people with severe influenza, and they suspect that the same mechanism may be responsible for bad outcomes in a range of other viral diseases as well.

"We will definitely know more soon," Meyts says.

In the meantime, other CHGE scientists are looking at the flip side of severe COVID: the possibility that some people carry gene variants that protect them from the disease. It's an intriguing idea but very hard to corroborate.

Anecdotally, however, the world is full people who've repeatedly been exposed but never been infected. "There are health care workers who worked in the ICU during the height of the pandemic whose tests kept coming back negative," says András N. Spaan, a former postdoc in Casanova's lab who now heads his own research team at University Medical Center Utrecht in the Netherlands. "And there are couples where one partner was sick and the other remained healthy even though the two lived together without isolating."

Are these cases mere flukes, or could it be that some people's genetic makeup makes them invincible to the virus—not only symptom-free but also unable to infect others? Spaan is conducting a study to find out.

The project faces distinct challenges. For one thing, there is little precedent for genetic superpowers in the context of infection. Spaan knows of just a few examples, including reported cases of people resistant to HIV,

malaria, or norovirus. And recruiting volunteers can be tricky. "People typically show up in the clinic because they're sick," Spaan says. "Those who stay healthy are less likely to encounter specialist physicians who would enroll them in a study."

Nonetheless, he and Casanova have heard from thousands of people who offered to participate in the research, suspecting that they might be COVID-resistant. "Our work has received a lot of helpful press coverage," Spaan notes. However, he says less than 10 percent of volunteers meet the recruitment criteria, which seek to ensure that participants have in fact been exposed to the virus. "It's one of our biggest challenges," Spaan says. "We're trying to distinguish between those who may be genetically resistant and those who didn't get sick for other reasons: maybe they were vaccinated, wore a face mask, or took other precautions."

A COVID-resistant individual would have gotten rid of the virus very quickly, either because it failed to enter host cells or because the person's immune response immediately overpowered it.

"It's very hard to prove that something was there at one point if it's no longer there," Spaan says.

The researchers are following several leads, including one emerging from another line of investigation focused on what may be the pandemic's weirdest clinical manifestation: pernio, or "COVID toes." Before SARS-CoV-2 came along, Ahmad Yatim, a dermatologist at Lausanne University Hospital, would occasionally see patients come in with discolored, swollen toes or fingers. These cases peaked dramatically during the first and second COVID wave, leading to the widespread assumption that pernio is a symptom of the disease. To their surprise, however, Yatim and his colleagues found the opposite. Most patients with COVID toes tested negative by PCR as well as by antibody testing, suggesting they were free of COVID and hadn't had it in the past either.

"We've come to think that pernio is indicative of a very strong interferon response that instantly shuts down the virus," says Yatim, who is currently conducting postdoctoral research in Casanova's lab.

The theory makes intuitive sense: If some people get sicker than others because their interferon response is inadequate, why wouldn't there be others capable of churning out so much interferon that the virus has no chance at replicating? The researchers believe most people fall somewhere in between these extremes, and the better your interferon response, the milder your COVID symptoms.

Still, the interferon-spectrum model remains to be verified. To that end, the team is looking for mutations that predispose to resistance. Spaan suspects such mutations might be very rare—but if they can be found, the impact would be huge.

"Inborn errors are experiments of nature," Spaan says. They make it possible to peek under the hood of an infectious disease and start doing what centuries of pathogen-centric work has often failed to do: untangle the disease mechanisms at play in human cells and tissues. And although inborn-resistance mutations will be much harder to find than bona fide "errors"—mutations linked to poor outcomes—they tend to be more effective in pointing the way toward the development of new therapeutics.

"The genetics of resistance is a best-case scenario in which the body fights off pathogens very efficiently," Spaan says. "It can directly show us how to confer powerful immunity to people who don't naturally have it." ◎

"The genetics of inborn resistance can directly show us how to confer powerful immunity to people who don't naturally have it."

1 in 5

Estimated ratio of people aged 18 to 64 experiencing at least one medical condition due to COVID up to a year after infection. Among those 65+, the ratio narrows to 1 in 4.

INTERVIEW

Cryo-electron microscopy is revealing the forms and functions of proteins faster than ever. Jue Chen says it's already producing revelations that will strengthen medicine and drug design.

The shape of things to come

By Eva Kiesler and Jen Pinkowski

We tend to think of machines as being of human origin—objects made by us and for us and that wouldn't exist without us. But proteins mastered molecular machinery billions of years ago, long before the first tetrapod crawled onto land. In short order, protein machines began to operate all of life. We can talk, run, communicate, sleep, and think because proteins are doing all the heavy lifting and smooth automation necessary for cell function: pumping, transporting, linking, bonding, grasping, shunting, filtering, and disposing of materials.

But much about how proteins operate is still mysterious. As with any cleverly designed device, their function follows their form, and any given protein can take on several forms, or conformations. So with every new

Illustration by Ibrahim Rayintakath

conformation that the protein folds itself into comes a new function. To truly understand what it's up to, you have to see it in action.

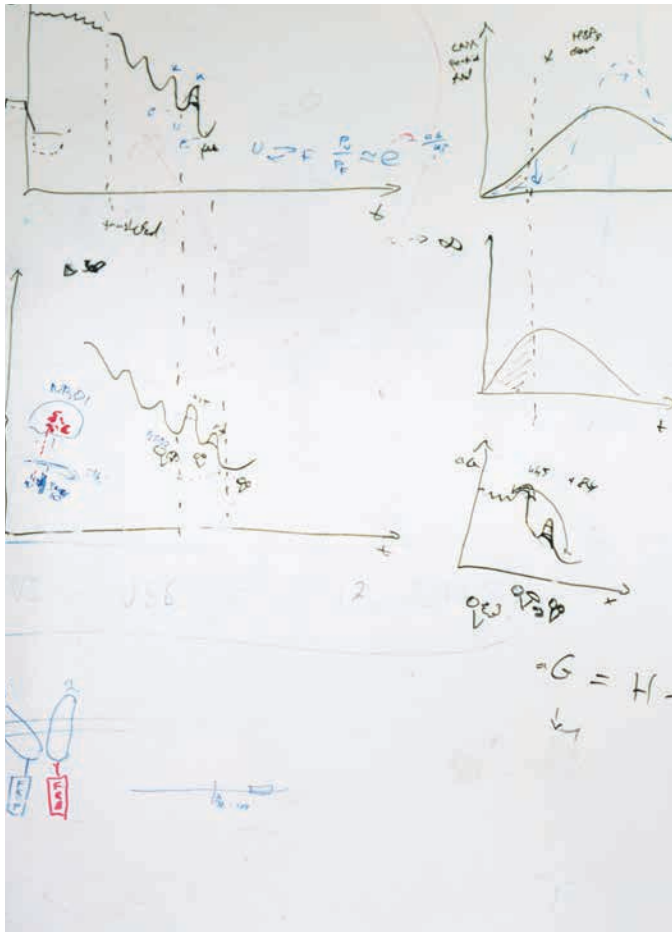
Take the maltose transporter protein complex, whose job it is to absorb sugar molecules into bacterial cells. It's been scrutinized for decades by structural biologists who hoped it would serve as a skeleton key to unlock the mysteries of all ABC transporters, molecular pumps that move cargo such as sugar molecules into or out of the cell. Its components had all been identified, but how they worked together as a functional unit eluded everyone—until Jue Chen, a specialist in ABC transporters, captured the first snapshots of the full maltose transporter in action using X-ray crystallography, a method in which molecules are painstakingly purified and crystallized and then studied with radiation.

It was an utterly revelatory moment for her. "It was beautiful; I could see every atom," says Chen, the *William E. Ford Professor* and head of Rockefeller's Laboratory of Membrane Biology and Biophysics. "It's incredible when a new structure gives you that intuitive understanding of how a process works. It's the only way to understand how biology happens at its most basic level."

Historically, such revelations about molecular architecture and dynamics have been hard won, with many scientists spending years or even a lifetime to solve the structure of a single protein. Many molecular components are too fragile for X-ray crystallography's dehydration process and simply collapse into chaos. But in the past decade, breakthroughs have been coming faster than ever for structural biologists like Chen thanks to a new technique called cryo-electron microscopy (cryo-EM). Insights that used to take years to emerge can now appear in months.

Not surprisingly, Chen's research has bloomed too. With new ways to watch molecular events up close, her lab is making swift progress in exploring ABC transporters, generating findings that might one day be used to prevent cancer cells from spitting out killer drugs or to treat diseases such as cystic fibrosis.

We asked Chen to tell us more about what this methodological revolution means for medicine and science, and what other advances we can anticipate.

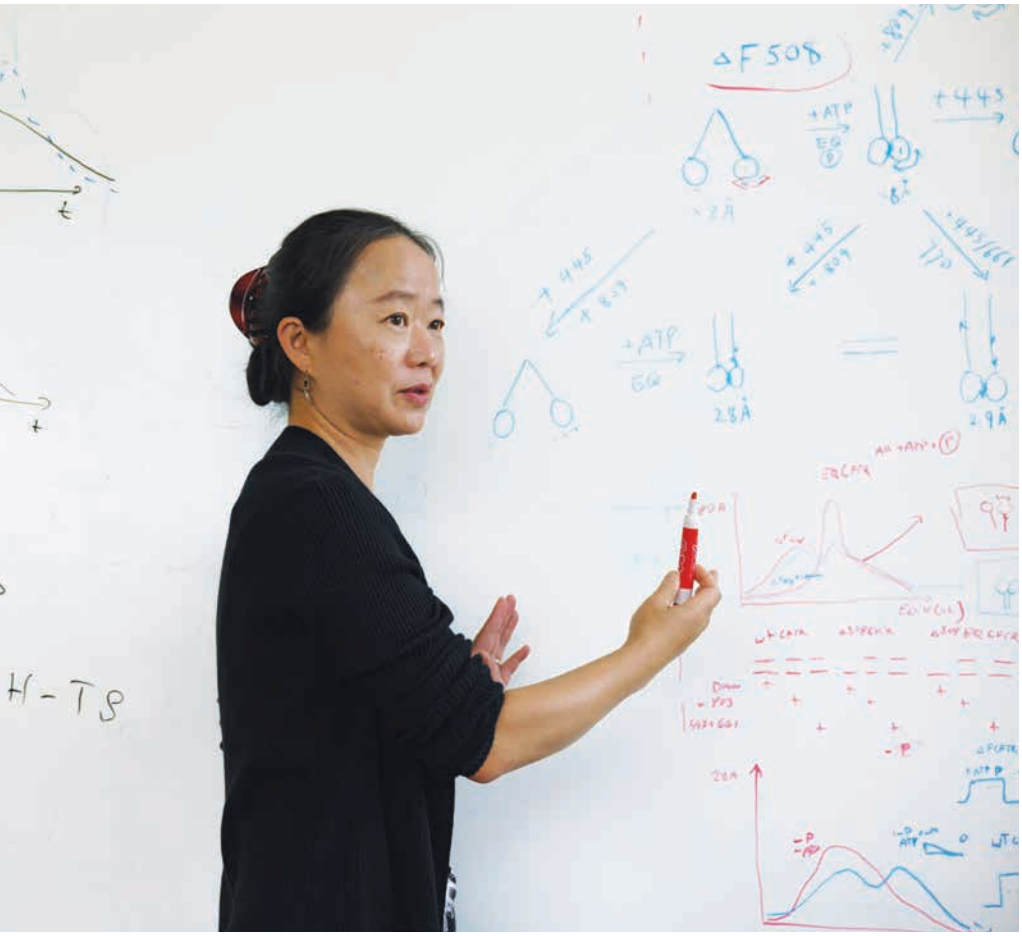


What was it like to be a structural biologist when you began your career?

The work was very different from what it is today. To solve the structure of a protein, we had to purify it and make it form a crystal that we then probed with X-ray radiation. And while a simple compound like table salt will easily arrange itself into a neat lattice, biomolecules tend to be flexible and fragile, making the crystallization process incredibly demanding and time-consuming. To succeed, you had to be a very good biochemist—and you had to be extremely patient and careful.

I've spent years in the lab trying and failing to grow crystals from the molecules I wanted to study. We used high-throughput technology and tried thousands of conditions at a time. We tried different chemicals. We tried modifying the protein to hit the right spot. It could take many, many years and thousands and thousands of trials.

There have been many proteins I was very interested in that never crystallized. This



Chen's team discovered a new drug mechanism that she hopes can be employed against hundreds of diseases.

CLAIRE HOLT

was the case with CFTR, for example, a protein that moves chloride ions across the cell membrane and whose mutation is responsible for cystic fibrosis, a devastating lung disease. One piece of the protein responsible for regulating the channel is so floppy that crystallization is virtually impossible. I was never able to make it work.

That sounds frustrating. How did cryo-EM impact your research?

Cryo-EM solved our problems. When I joined Rockefeller in 2014, a team at the University of California, San Francisco had just published a landmark paper on the use of this technique for structural biology. Rockefeller immediately allocated funds to set up the instrumentation, and several of my faculty colleagues and I raced to learn the new technique ourselves. With cryo-EM, we no longer needed to grow crystals; instead we could suspend the molecule of interest in a buffer solution and flash-freeze it. This technique not only enables us to solve structures that were previously beyond reach but also makes it possible to simultaneously capture many snapshots of a molecule as it transitions from one state to the next, giving us unprecedented insight into its dynamics.

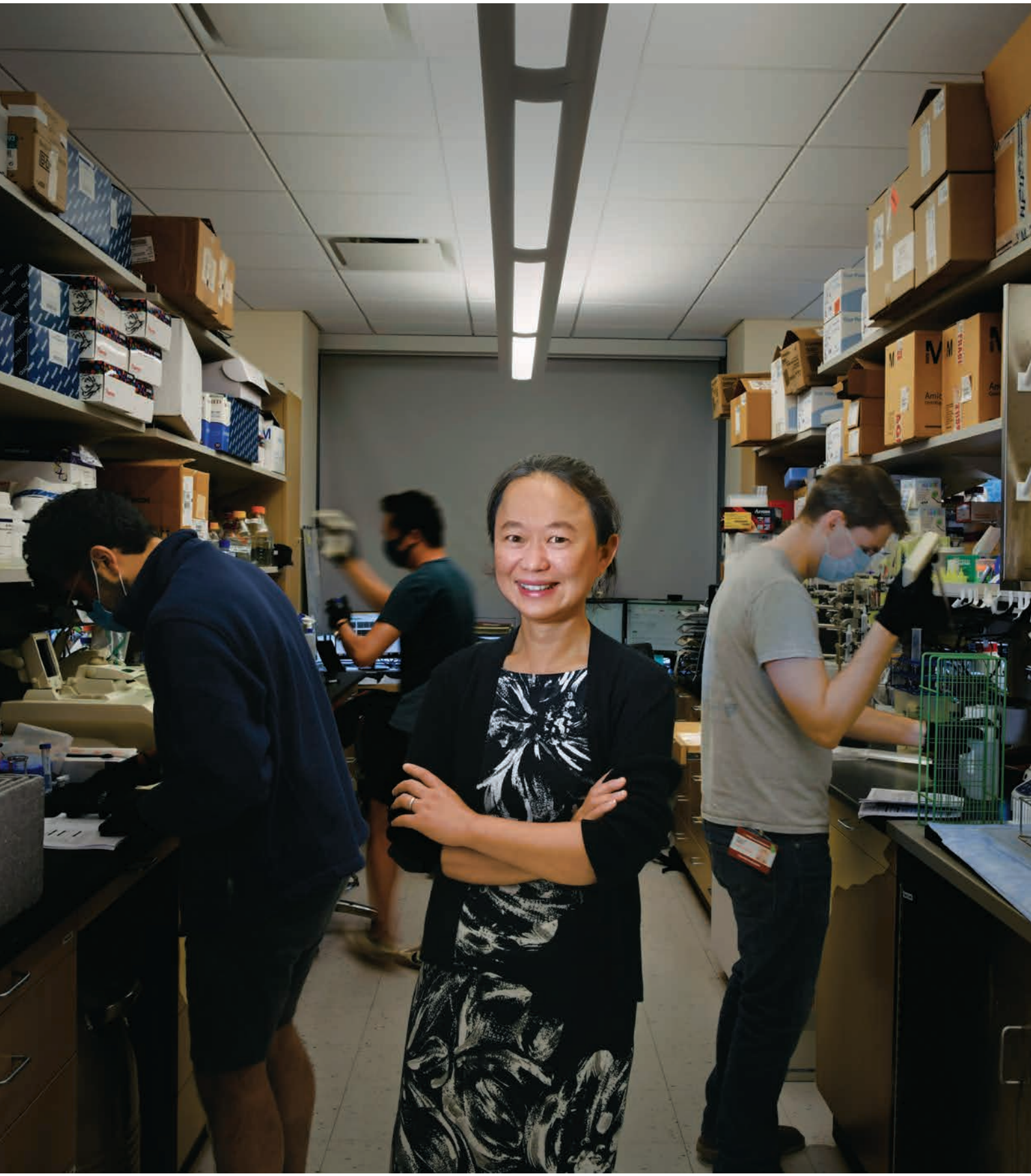
You basically take many 2D pictures of the purified molecule, and then you reconstruct its shape in 3D. With every step, the molecule is telling you, "This is what I look like." We also stitch together snapshots of the same machinery at different stages of its work and put them together as a movie.

We were just so excited because with all these projects we worked on for years, we couldn't get a hint of crystallization. Then boom, boom, boom—one after another, we solved them by cryo-EM. It felt so empowering.

How might this new knowledge improve medical treatments?

It's already beginning to enable new approaches like rational drug design—the idea of synthesizing drugs based on what we know about disease-related molecules and the way they bind with their molecular partners, or ligands. Now that it only takes a few months to produce structural renderings, we can look at a pair of interacting molecules and ask, "What will it take to make their binding more efficient? What if we add a certain chemical group at this or that location?" It means we will be able to iteratively optimize drugs to eliminate side effects, for example, or to improve their uptake in cells and tissues.

I'm very excited about computational "docking" techniques that make it possible to discover new ligands. Until now, most existing drugs have been identified by brute-force searches in which millions of molecules had to be tested. Scientists are developing increasingly smart software that can analyze the properties of molecules and predict the ones with the highest chance of forming a stable complex with a target—a mark of the molecule's potential as a future drug.



It may soon become possible to optimize drugs to eliminate side effects or improve uptake in cells and tissues.

Another big opportunity for drug discovery is to study the function of existing medications. We don't actually know how many of our best drugs work, and learning how one medication does its job might open the door to developing new ones based on the same principles.

Could you give us an example?

My lab recently solved the mystery of how CFTR correctors, a class of cystic fibrosis drugs, work. It turns out that the CFTR protein has an internal cavity that makes it intrinsically unstable. This defect is exacerbated in people with various kinds of mutations, preventing it from folding properly. As a result, it gets prematurely degraded inside the cell. We were able to understand how CFTR correctors fix this problem: They bind inside the cavity and fill it, stabilizing CFTR and prolonging its life span (read more about the lab's work on CFTR correctors in "How misfolded proteins get into shape," on page 12).

No other drug has previously been shown to act in this way, and we suspect the same mechanism could be employed to design new drugs against a range of diseases. Beyond cystic fibrosis, there are hundreds of illnesses believed to be caused by the inability to produce a correctly shaped protein, including neurodegenerative conditions like Parkinson's and Huntington's. Could these diseases also be treated by correcting a misfolded protein, and does that protein have internal cavities like CFTRs do? We are doing the experiments to find out.

Tell us more about how computational tools are advancing the field. What about AI, for example?

AI tools are making it possible to predict the structure of a protein from its amino acid sequence. Scientists have tried to make such predictions for decades based on the spatial configurations a particular stretch of amino acids will have due to its chemical properties. The new AI tools perform much better. They use machine-learning algorithms to sift through a database and pick the shape most likely to match a sequence.

But AI predictions are not always accurate, and they will never replace experimental techniques. Still, in some cases they can save a lot of time. People in the field increasingly rely on them to guide experiment design, for example.

So, what might be the next revolution?

Thus far, we've mainly been looking at proteins in isolation. We break the cell open, take the protein out, and work with it in vitro. But many proteins don't fold or function properly outside of their natural environment, so there's always a risk of collecting bad data.

In the future, emerging technologies will enable us to look at molecular structures in living cells—maybe even within living tissues. For example, we might soon be able to use cryo-EM to determine a protein's cellular distribution and watch it interact with other proteins in its surroundings, all while still being able to see its structure with near-atomic resolution. It will be amazing.

Do you ever get nostalgic for the old days of crystallography?

Sure—determining structures was very challenging back then, and I love a challenge. It's a very special feeling when you get to see something nobody has seen before, even more so if you've been working on it for years.

Still, it's hard to ignore that cryo-EM has made our day-to-day work more interesting and versatile. It has freed up so much of our time, giving us the opportunity to ask more complex questions and do all kinds of biological experiments. Everyone in my lab has a structural biology component, but they do many other things as well. Now we are looking for molecules that regulate the function of a protein, making it more active or inhibiting its function. To be honest, our field is more fun now than it's ever been!

CLAIRE HOLT

A scent paradise for flies

NOTHING GETS *Drosophilae* going like the tangy perfume of apple cider vinegar, which evokes the scent of rotting fruit. From atop a spinning ball at the center of a virtual reality setup, the insect will run or fly toward the scent while Chad Morton and Andrew Siliciano, both graduate students in Vanessa Ruta's Laboratory of Neurophysiology and Behavior, observe the neural activity enabling it to navigate an aroma-rich world.

To craft this "odorverse," the scientists retrofitted a fly treadmill system developed in the lab of Gaby Maimon with a 3D-printed plate that allows the fly to rotate as it walks about its fictive environment. Working closely with engineers in the Precision Instrumentation Technologies shop, they then added a pistol-shaped nozzle for odor release that connects to airflow-modulating controllers and scent vials. "We used Python to program the software that manipulates the odor dispenser," says Morton. "That way," adds Siliciano, "we can precisely measure the amount and vary the concentrations of the odor we're releasing." (In between experiments, they might expel a puff of 1-octanol acid, a palate-cleansing neutral fragrance.)

Until recently, the only way scientists could manipulate individual scents was to turn them on or off. But the high-precision scent diffuser makes it possible to more accurately simulate how a fly might navigate amid multiple smells, so scientists can explore what happens when it loses a scent trail and the decisions it makes along the way.

These experiments are already revealing that a fly's path to an aroma is more elaborate than previously thought. Rather than tracking straight up the center of a plume, it prefers to wiggle in and out of the plume's periphery, perhaps taking a whiff of surrounding scents to stay open to other environmental inputs.

And while the fly's goal may be to land on a juicy grape, Morton and Siliciano are after something more elusive: the circuits that fire as the fly smells its way around—intel that might tell us more about the basic functioning of sensory systems.

Photograph by Matthew Septimus

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