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M E D I C I N E

Issue 1 / 2022

special report

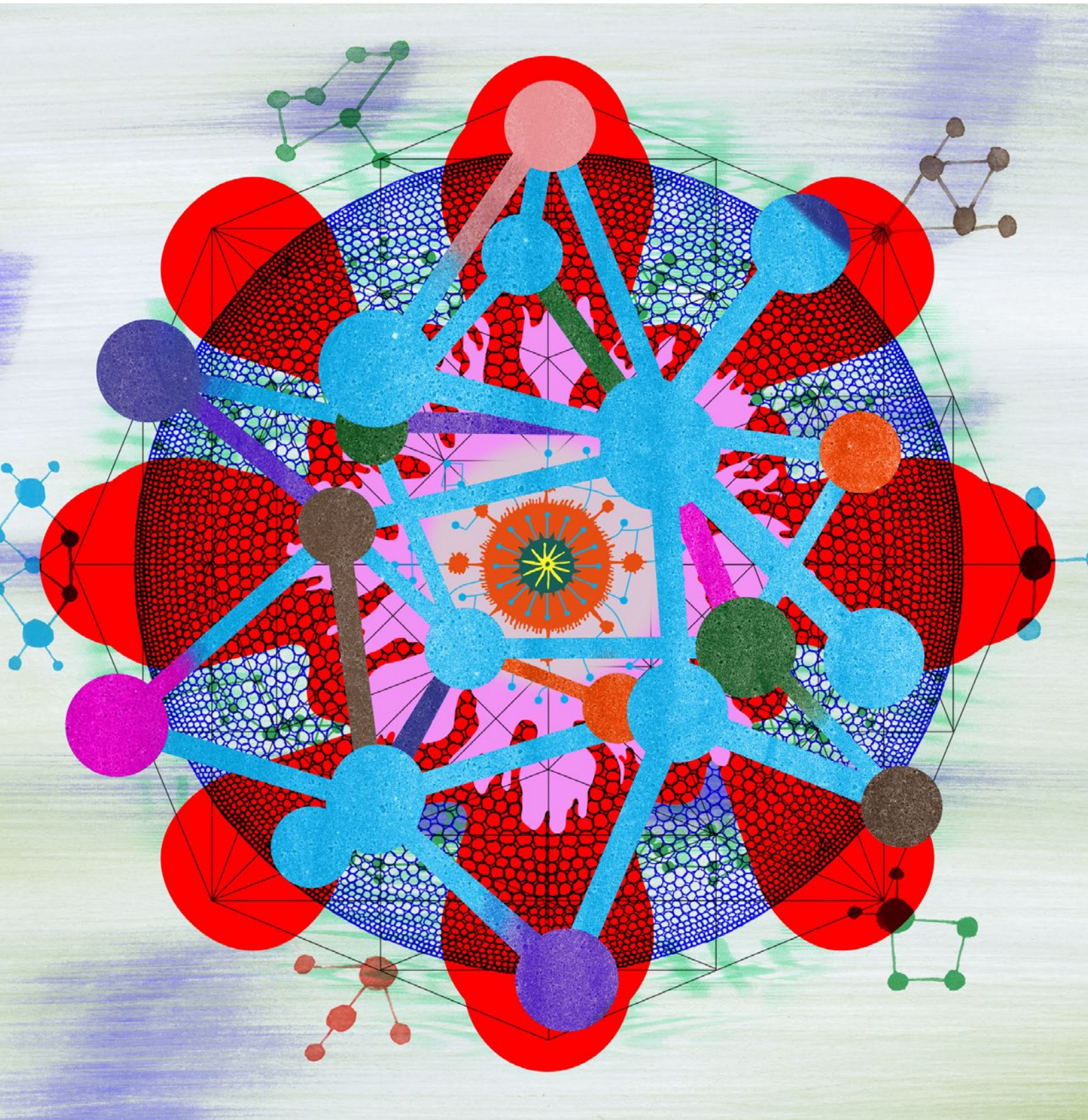
MOLECULES OF LIFE

Understanding the world within us

plus

STANFORD MEDICINE

Issue 1 / 2022



PUTTING PATIENTS FIRST IN PRESCRIPTION OPIOID REGULATION

CHIEF OF COMMISSION ON NORTH AMERICA'S OPIOID CRISIS REFLECTS ON THE IMPORTANCE OF CONSENSUS

By Keith Humphreys

When I agreed to lead the Stanford-*Lancet* Commission on the North American Opioid Crisis, I knew I was striding into a combat zone. For the past quarter century, the medical community — as well as the rest of the country — has formed competing camps that emphasize either the destructive power of opioids or their therapeutic usefulness. Our commission's model estimated that if we don't change our current policies, over a million people will die of opioid overdoses in the United States this decade. The more the former camp highlights the potential of prescription opioids to cause addiction and overdose, the more the latter camp highlights how underprescribing opioids harms those suffering chronic pain.

Keith Humphreys, PhD, the Esther Ting Memorial Professor in the Department of Psychiatry and Behavioral Sciences at Stanford Medicine, chairs the Stanford-*Lancet* Commission on the North American Opioid Crisis.

These dynamics, to some extent, reflect shortcomings in how we judge risk and benefit. Psychologists, including Paul Slovic, PhD, a national expert on decision making and risk analysis, have shown that we tend to mentally outsource judgments that should involve complex reasoning about risks and benefits to simple emotional responses. If we have a good feeling about the benefits of something (That car looks fun to drive!), we tend to minimize its risks (It has a terrible crash record). If we have a negative feeling about the risks, we tell ourselves the benefits are exaggerated.

In reality, high risk and high benefit can work together, as can low risk and low reward. I didn't want the commission recommendations to fall into this mental trap by painting opioids as either a menace or a panacea. I've spent my career in the addiction field, where the harms of opioids are very evident, but I've also spent a decade volunteering as a counselor in hospice, where the benefits of opioids are very evident.

A radio show producer who was arranging a panel on the opioid crisis asked me, "To ensure balance during the debate, I just have to ask if you are for or against opioids." I responded, "No." Needless to say, they found another guest.

I am proud of the justice done by commission members to addressing the complex nature of the opioid crisis — recommending policies to reduce corporate overpromotion of opioids, while recognizing the need for medical schools to teach students about the many effective uses of this class of medication.

How was this consensus achieved? And how can I promote a similarly nuanced stance in my teaching and interactions with policymakers? I haven't cracked the mystery, but I've learned something valuable.

The entire ecosystem around the opioid crisis separates people into competing factions. This includes formal structures, like journals and professional societies, and informal ones like Twitter. When a group interacts only with its own members, judgments and hostilities toward those in other groups tend to become more unbalanced and extreme.

The commission included people who wouldn't normally be in the same room: experts in addiction, pain medicine, law, and public policy, as well as people with lived experience of addiction and chronic pain. Yet they found a way to listen to each other. It was candidly hard work at times, certainly more so than if everyone had huddled only with their own. It might take that combination of diverse perspectives and experiences, combined with an ethic of collegiality, to break out of our simplistic viewpoints about opioid use and addiction (and perhaps for many other issues as well).

"We should not be pro-opioid or anti-opioid," said commission member Sean Mackey, MD, PhD, chief of the Division of Pain Medicine and the Redlich Professor at Stanford. "We should be pro-patient." **SM**

TIMOTHY ARCHIBALD

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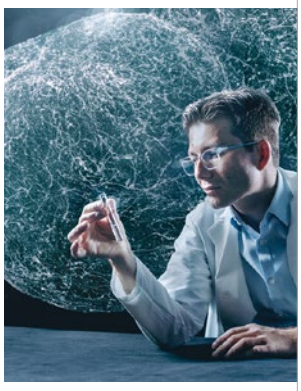

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letter from the dean

Fundamental research is, fundamentally, an act of exploration. It is the pursuit of knowledge in its purest form — an endeavor to unravel the biological mysteries of our world and shed light on the building blocks of life.

Driven by a desire to expand humanity's knowledge, Stanford Medicine's basic scientists have produced some of the most transformational biological breakthroughs of the past seven decades. The origins of this success trace back to the School of Medicine's migration from San Francisco to The Farm in 1959. The 35-mile journey to Palo Alto has pushed us further than we could have imagined, propelled by the interdisciplinary collaborations that have flourished between the School of Medicine and schools across the university.

The effort to enhance and deepen our research involved more than relocation, however. Stanford recruited world-class scientists to its new campus, including Nobel laureates Arthur Kornberg, MD, and Joshua Lederberg, PhD. Dr. Kornberg was recognized in 1959 for his discovery of DNA polymerase, an enzyme essential for DNA replication, while Dr. Lederberg was honored in 1958 for his work focused on genetic recombination and the organization of the genetic material of bacteria.

The legacies of Dr. Kornberg and Dr. Lederberg and countless other groundbreaking researchers from the annals of Stanford's history live on today. Stanford's School of Medicine has seven Nobel laureates on its faculty and legions of researchers defining the future of biomedicine through their discoveries. Perhaps most telling about the quality of Stanford's fundamental research is that our school consistently has the highest National Institutes of Health funding per faculty ratio in the country.

Indeed, Stanford's basic scientists have made extraordinary contributions to biomedicine. Their hard-earned discoveries often open whole new fields of study. And with every advance, they forge new pathways for future discoveries.

Consider Stanford professor Brian Kobilka, MD, who received the Nobel Prize in 2012 for his studies of G-protein-coupled receptors — a critical target for over a third of approved drugs, including morphine and other painkillers. Building on this groundbreaking work, Dr. Kobilka had a lead role in discovering a novel compound that may be as effective as morphine for pain relief while causing less respiratory suppression, an adverse side effect that contributes to 30,000 overdose deaths in the United States annually.

This is the potential of basic science, and more promising work is on the horizon. Among many other extraordinary examples, last fall, Stanford neurologist Michelle Monje, MD, PhD, received a MacArthur Fellowship, commonly known as a genius grant, for her pioneering work in the emerging field of cancer neuroscience. Her research has deepened our understanding of how the brain's glial cells support neurons and how cancers attack them.

As Stanford's legacy of scientific discovery has demonstrated, basic research is continually evolving. In the process, it changes not only how we see the world around us but also how we live in it.

I'm proud that this latest issue of *Stanford Medicine* magazine celebrates fundamental research and explores the golden age of discovery that we find ourselves in today.

I believe we will look back on this time with immense appreciation for the limitless curiosity of our basic science trailblazers for decades to come. I look forward to seeing how their pursuit of knowledge will generate new discoveries — and fundamentally change our understanding of life as we know it.

Sincerely,
Lloyd Minor, MD
Carl and Elizabeth Naumann Dean of the School of Medicine
Professor of Otolaryngology-Head & Neck Surgery

GLENN MATSUMURA

upfront

A QUICK LOOK AT THE LATEST DEVELOPMENTS FROM STANFORD MEDICINE

Improving colitis treatment

CHOLESTEROL-LOWERING STATIN DRUGS could provide significant relief for the nearly 1 million people in the United States with ulcerative colitis, a new study showed.

Ulcerative colitis causes inflammation and ulcers in the bowel, leaving patients vulnerable to extreme abdominal pain, blood in the stool, constipation and fatigue, said senior author Purvesh Khatri, PhD, an associate professor of medicine and of biomedical data science.

The condition is often debilitating and has no real cure. Treatments include anti-inflammatory and immune-regulating drugs. Another option is surgical removal of parts of the colon.

"It's a drastic measure," Khatri said. "So we thought, 'Can we use available data to see whether drugs that are already approved by the FDA can be repurposed to better treat these patients?'"

His team analyzed publicly available anonymized patient health information that included genomic and prescription data and searched for FDA-approved drugs that reversed gene activity patterns in ulcerative colitis patients.

MELINDA BECK

People with ulcerative colitis who were taking statins had about a 50% decrease in colon surgery rates, were less likely to be hospitalized and were prescribed other anti-inflammatory drugs at a lower rate, revealed the study, published in September 2021 in the *Journal of the American Medical Informatics Association*.

"I think we're almost there," Khatri said. "We need to validate the effects a bit more stringently before moving it into the clinic."

Without intervention, the opioid epidemic could kill **1.2 million people** in the U.S. this decade. More at stan.md/opioids.

Say, what?

MOMS, LISTEN UP: If your 13-year-old appears to hear only, "Wah wah," when you speak, don't worry.

That's just her brain helping her to start separating from you by turning down your voice and turning up unfamiliar voices, Stanford Medicine research published in April in the *Journal of Neuroscience* found.

"This is a signal that helps teens engage with the world and form connections which allow them to be socially adept outside their families," said the study's senior author, Vinod Menon, PhD, the Rachael L. and Walter F. Nichols, MD, Professor of psychiatry and behavioral sciences.

Read more: stan.md/MomsVoice.

Depression relief

A new type of magnetic brain stimulation eliminated symptoms in more than three-quarters of severely depressed people participating in a Stanford Medicine study.

"It works well, it works quickly and it's noninvasive," said Nolan Williams, MD, an assistant professor of psychiatry and behavioral sciences and senior author of the study, published in October 2021 in the *American Journal of Psychiatry*.

There were 29 people with treatment-resistant depression in the study. About half received the new treatment — Stanford accelerated intelligent neuromodulation therapy, known as SAINT. The rest were given a placebo treatment.

After five days, 78.6% of the treatment group participants were no longer depressed. "It's quite a dramatic effect, and it's quite sustained," said Alan Schatzberg, MD, the Kenneth T. Norris, Jr. Professor in Psychiatry and Behavioral Sciences, who was a co-author of the study.

Tommy Van Brocklin, 60, was depressed for 45 years and unable to find lasting effective remedies. Soon after the new treatment, he was able to make major life changes. "I'm sleeping better. I completely quit alcohol," he said. "I'm walking my dog and playing the guitar again, for nothing more than the sheer joy of it."

Growing heart parts

IN THE PAST, INFANTS WITH SEVERE HEART ABNORMALITIES OFTEN DIDN'T SURVIVE THE SURGERY meant to save them. Medical advances have improved odds for the about 40,000 babies born in the U.S. every year with heart defects, including an extra hole in the organ or a single ventricle instead of two. But even successful surgeries can compromise their health and activities.

Hoping to help these children, Mark Skylar-Scott, PhD, an assistant professor of bioengineering, is creating heart tissue in the lab using 3D-printing techniques.

The process starts by coaxing stem cells — cells that can give rise to other cell types — to become the cells that power the heart's contractions and the cells that comprise the heart's connective tissue. By layering the cell types, the scientists create organoids, clumps of the tissue designed to mimic healthy heart tissue.

The team has already grown a 2-inch-long veinlike tube that can contract and expand to move fluid through itself. In theory, it could help those born with only one ventricle send blood from their heart to the rest of their body, Skylar-Scott said.

Much more research is needed before the engineered tissue can be tested in clinical trials, but Skylar-Scott is optimistic about the possibilities.

"Once the pipeline for new cells is in place, I think we're going to start seeing some incredible progress," he said.

MELINDA BECK

COVID-19 in overdrive

AT LEAST 1 IN 5 **hospitalized** COVID-19 patients develop new antibodies that attack their own tissue within a week of admission, a Stanford Medicine-led study shows.

"If you get sick enough from COVID-19 to end up in the hospital, you may not be out of the woods even after you recover," said PJ Utz, MD, professor of immunology and rheumatology and co-senior author of research published in September 2021 in *Nature Communications*.

The rogue attackers, called autoantibodies, could result from immune-system overdrive triggered by a virulent, lingering infection, researchers said. The abundance of cytokines — proteins the immune cells rally to fight infection — may trigger the erroneous production of antibodies targeting them, Utz said.

Vaccinations, he added, decrease the likelihood the immune system will be confused into generating autoantibodies.

ADHD TREATMENT GAP

THOUGH PARENT TRAINING in behavior management is the recommended starting point for treating 4- or 5-year-old children with ADHD, few pediatricians start there, research published in October 2021 in *JAMA Pediatrics* shows.

Lead study author Yair Bannett, MD, a developmental and behavioral pediatrician at Stanford Children's Health, said the behavioral therapy acknowledges the challenges of parenting ADHD children and provides helpful tools — such as teaching parents to reward their children's good behavior, set appropriate limits and consequences, and develop predictable daily routines.

Studying 22,714 charts of 4- and 5-year-old children, researchers identified 192 with attention-deficit/hyperactivity disorder symptoms or a diagnosis, finding that behavior therapy was recommended for only 11% of those children.

Medication was prescribed in 17% of the cases, which Bannett said is rarely appropriate for children that age.

Brain cells gone bad

THE LATE STANFORD MEDICINE NEUROSCIENTIST Ben Barres, MD, PhD, was among the first to discover that a certain type of cell in the brain is far more instrumental in the shaping and care of neurons than had previously been imagined.

But under some inflammatory conditions, Barres' team reported before he died in 2017, these benevolent cells, called astrocytes, change course and destroy injured neurons that might have survived. The team suspected the destruction was caused by an unknown substance that astrocytes oversecrete when they go rogue. At the time, Barres said discovering how the astrocytes go wrong could be key to halting the progression of such neurodegenerative disorders as Lou Gehrig's, Alzheimer's, Parkinson's and Huntington's diseases.

Barres said discovering how the astrocytes go wrong could be key to halting the progression of such neurodegenerative disorders as Lou Gehrig's, Alzheimer's, Parkinson's and Huntington's diseases.

Though they are still looking for the mechanisms behind the phenomenom, Kevin Guttenplan, PhD, a former Barres grad student, and Shane Liddelow, PhD, a former postdoctoral scholar in Barres' lab, have identified the toxins astrocytes use as ammunition when they turn bad.

Guttenplan, now a postdoc at Oregon Health and Science University, was lead author of the research published in October 2021 in *Nature*. Liddelow, now an assistant professor of neuroscience and physiology at New York University, shared senior authorship with his deceased mentor, Barres.

"Initially, everybody assumed the mystery toxin was probably a protein," said Guttenplan. But it turned out to be certain unusually elongated lipids, carried by the protein ApoE, which regulates fat metabolism. These lipids, which are more deadly as they grow, are normally produced at low levels. But production jumps when astrocytes behave badly. "This was our homage to Ben," said Liddelow, who is pursuing a drug to block the enzyme that's essential to producing the extra-long lipids.

Of mice and men and women

NEW RESEARCH POINTS to significant differences in gene-activated sexual behavior of male and female mice, leading scientists to speculate that the same could be found in humans. The scientists, led by Nirao Shah, MBBS, PhD, professor of psychiatry and behavioral sciences and of neurobiology, probed four tiny structures within mouse brains that program "rating, dating, mating and hating" behaviors.

The behaviors — such as males' quick determination of a stranger's sex and females' mating receptivity and maternal protectiveness — help the mice reproduce and their offspring survive. Analyzing tissue taken from these brain structures and enriched for cells responsive to sex hormones, researchers found more than 1,000 genes to be much more active in the brains of one sex than the other.

"Using these genes as entry points, we've identified specific groups of brain cells that orchestrate specific sex-typical behaviors," Shah said. The study, published in January in *Cell*, helps explain sex differences in mammals.

HUMAN BIOLOGY AT ITS MOST BASIC

Molecular insights boom

KRISTA CONGER
ILLUSTRATION BY JEFFREY DECOSTER
PORTRAIT BY TIMOTHY ARCHIBALD

Look around you.
Nearly everything you see is made up of molecules.

Your hand, the wall, the paper page on which you might be reading these words. Heck, even the thoughts that are forming in your head as you scan this sentence are manifested by the release of neurotransmitters (molecules!) scampering across the synapses between your brain's neurons (cells, which — spoiler alert! — are made up of clumps of molecules working in synchrony to carry out the busy business of life).

But what are they, actually? Molecules are formed by atoms — remember the periodic table? — that clasp each other tightly in ways dictated by the capricious orbits of their electrons and the relative numbers of their protons, neutrons and electrons. In doing so, they create magic. They blossom into more than the sum of their parts, becoming water, oxygen, even the genetic material that makes you, you.

But sometimes these tiny structures go awry. A change in the net electrical charge of the hemoglobin molecule that ferries oxygen from the lungs predisposes a person to a lifetime of sickle cell anemia; a missing building block in a molecule that controls the flow of salt and fluids across cellular membranes causes the

buildup of thick, sticky mucus in the lungs of people with cystic fibrosis; a swap of a nucleic acid near a gene that controls how, when or how often a cell divides leads to an uncontrollably growing tumor. And sometimes, a molecule made by a virus new to humans binds to other molecules on the surface of respiratory cells and, in the blink of an eye, launches a pandemic that is still raging across the world.

Understanding how molecules function in living organisms, and the health consequences of their failures, is the bedrock of what is still a relatively new field of science — molecular biology.

Recently, our ability to conduct such studies has catapulted forward with the development of new visualization technologies such as cryo-electron microscopy, the expanding computing capabilities available to biologists, and the development of new techniques to explore not just a molecule's structure but also its neighborhood, identifying working groups and cliques that make a cell tick in a particular way in specific circumstances. These advances are further illuminating the secret lives of molecules — peering behind the curtain, under the sheets and in the closets — in ways that are expected to revolutionize how medicine is practiced.

"We are at an extremely important point in scientific history," said Ruth O'Hara, PhD, senior associate dean for research and Stanford Medicine's Lowell W. and Josephine Q. Berry Professor. "Molecular medicine is a vast domain that spans from basic science research aimed at understanding the molecular basis of diseases, to identifying potential therapeutic targets, to preclinical and clinical trials of new drugs. Mining complex molecular data and overlaying them on clinical outcomes is critical for precision health and medicine, and Stanford Medicine excels at it. This is a special place."

Stanford has stood out among its peers since the medical school moved from San Francisco to Palo Alto in 1959 to cultivate the training of a rare breed of physician-scientists skilled not just in clinical care but also in the research techniques necessary to understand the causes of disease at the most basic biological level. Some recent returns on this approach: Stanford researchers have cracked the code of vicious DNA circles that enable cancer cells to evade common treatments, plumbed the sticky consequences of too much mucus throughout the body (and how to combat it), and grappled with the need for an effective, nonaddictive painkilling molecule.

Taking the mystery out of molecules

TO UNDERSTAND MOLECULES, you have to know something about atoms, which were imagined as far back as the fifth century by Greek philosophers who believed the universe is made up of infinitesimally tiny particles. They arrived at this conclusion by logicking their way to the idea that any substance, when divided in half, eventually reaches a state of being where it is impossible to divide it any further. (Think of striving to share a chocolate bar equally among hundreds of people in an office building.) They coined the remaining particles "atoms."

Molecules, the philosophers surmised, are made up of two or more atoms tightly entwined, perhaps by a hook-and-eye-type fastener. (Today chemists call these relationships covalent bonds.) While embracing, the atoms assume new chemical and physical properties by virtue of their now shared electrons. Hydrogen and oxygen, left to their own devices, are odorless, colorless gases. Together they become watery — quenching our thirst, keeping our iced tea cold and helping plants grow. Molecules are the smallest combination of atoms that maintain a material's physical and chemical properties (when is that office chocolate bar no longer chocolate?).

Scientists have been fascinated with molecules for hundreds of years. Like Lego pieces, molecules combine in countless ways to build macromolecules like DNA, proteins and structural components of cells carrying out the machinations of life. But molecules existed long before life itself. In

STANFORD RESEARCHERS HAVE CRACKED THE CODE OF VICIOUS DNA CIRCLES THAT ENABLE CANCER CELLS TO EVADE COMMON TREATMENTS, PLUMBED THE STICKY CONSEQUENCES OF TOO MUCH MUCUS THROUGHOUT THE BODY (AND HOW TO COMBAT IT), AND GRAPPLED WITH THE NEED FOR AN EFFECTIVE, NONADDICTIVE PAINKILLING MOLECULE.

2019, planetary scientists reported the discovery of helium hydride in a distant nebula called NGC 7027. Formed a mere 100,000 years or so after the big bang, this first-ever molecule is nearly 14 billion years old. It arose when the intense heat and pressure of the earliest days of the universe smashed together one hydrogen atom and one helium atom to form the first molecule.

Researchers had speculated about the existence of helium hydride in nature since it was first observed in a laboratory in 1925. But observing it in the wild took the development of specialized infrared viewing technology nearly 100 years later, as well as a way to send that technology high into the stratosphere to evade atmospheric interference that would drown out the signal from the elusive molecule.

Molecular biologists faced a similar problem in the mid-1900s when geneticists studying the mechanisms of inheritance in plants, fruit flies and viruses that infect bacteria realized they'd come to the limits of what they could understand with their "if this, then what?" observation-based experiments. They needed to see the stagehands behind the curtain: the molecules themselves. But to do so, they had to enlist the expertise of structural chemists, quantum physicists and crystallographers familiar with the techniques to study life at an atomic, or even subatomic, level.

X-ray crystallography — a technique in which molecules are coaxed to crystallize and are then bombarded with X-rays that ricochet off nuclei and barrel through electron orbits in a way that allows scientists to determine their structure — was the first technique to crack the three-dimensional structure of biological molecules such as cholesterol, penicillin and myoglobin. Nuclear magnetic resonance spectroscopy, which zaps molecules in a magnetic field with radio waves, and electron microscopy, which uses beams of electrons to illuminate the structure of microorganisms, cells and molecules, have delivered behind-the-scenes glimpses at worlds only dreamed of by biologists 100 years ago. But each technique has its limits, and many questions remained unanswered.

One of the newest advances is a type of imaging platform called cryogenic electron microscopy, or cryo-EM. Developers of cryo-EM were awarded the 2017 Nobel Prize in Chemistry, and Stanford's recent investment in five new cryo-EM machines establishes the university as a top center in the technology. Cryo-EM does more than just identify previously elusive molecules, however. It can also be used for the study of diseases and drug development — precisely what the early proponents of the medical school move had envisioned.

Redefining the medical school, refining molecular research

In the mid-1950s, Stanford University president Wallace Sterling and provost Frederick Terman were campaigning to shake up the medical school. At a time when the fashion was to streamline medical training to train more doctors more quickly, they wanted something that would set Stanford apart: a five-year program that encouraged budding physicians to spend an extra year researching a topic of their choosing.

"Our first principle was that Stanford was going to be a completely research-oriented medical school," the late Avram Goldstein, MD, recalled in 2000 in a *Stanford Medicine* article commemorating the 40th anniversary of the move. Goldstein, then-chair of Stanford's pharmacology department, recruited leading basic scientists and clinical researchers to Stanford. "It was a great challenge — and fun."

The result, they hoped, would be hybrid physician-scientists well-equipped to merge the fields of clinical care and basic research — research that could lead to medical discoveries. But to do so, the trainees needed laboratories and mentors familiar with more than stethoscopes and scalpels.

In the migration south in 1959 the medical school integrated more closely with the university's scientific departments and encouraged the crosstalk necessary to spark interdisciplinary collaborations. It also allowed the school to recruit Arthur Kornberg, MD, and six of his colleagues from Washington University in St. Louis, including Paul Berg, PhD, to establish a new department of biochemistry — the study of the chemistry of life. Kornberg was a top researcher in the burgeoning field. With noted geneticist Joshua Lederberg, PhD, who joined Stanford from the University of Wisconsin to launch a department of genetics, the researchers transformed the medical school.

"I vividly recall our first class," Berg said in an article about the early days of the biochemistry department. "Sixty students had enrolled, but the room, which seated 120, was jampacked." Berg would go on to share the 1980 Nobel Prize in Chemistry for his research on the biochemistry of nucleic acids and recombinant DNA.

Stanford has since become a force in merging basic research — looking into and beyond the microscope at the most basic chemical reactions and building blocks of life — and translational medicine — the purposeful effort needed to shepherd findings born on a laboratory bench into the clinic to help patients.

What started as an aspirational new approach to medical training is still evolving in the form of Stanford's recently launched Innovative Medicines Accelerator and its emerging Future of Life Sciences Initiative, which will enhance collaboration across the university and with the research and technology powerhouses in Silicon Valley and beyond. The emphasis on raising up the next generation of stellar clinician researchers remains in the form of Stanford's long-standing Medical Scientist Training Program, which allows students to simultaneously obtain a medical doctorate and a research doctorate in a six- to eight-year period of intense learning.

"Stanford School of Medicine reinvented itself more than six decades ago when it moved to the Palo Alto campus," said Lloyd Minor, MD, dean of the School of Medicine. "Since then, the school has been recognized with eight Nobel Prizes for transformative basic research that has had a direct impact on human health. These new initiatives build upon that strong foundation and extend beyond it to more effectively translate promising discoveries from the laboratory bench to the clinic while also promoting diversity, inclusion and health equity in the medical and research fields and in the communities they serve."

Lederberg already had his Nobel Prize when he arrived at Stanford in 1959; Kornberg was awarded his in 1959, the year of the move, and Berg received his in 1980. In the subsequent years, Steven Chu, PhD; Andrew Fire, PhD, Brian Kobilka, MD; Roger Kornberg, PhD; Michael Levitt, PhD; and Thomas Südhof, MD, PhD, would join the ranks of Stanford medical school faculty honored with the prestigious award. Each of these had his own research specialty, but they shared a common theme: the study of molecules.

It's not always enough just to visualize molecules in never-before-seen detail, however. It's also important to suss out what the little rascals are up to. Who do they hang out with, and when? What turns them on, or off? How can we distinguish the bad actors from the good? Sometimes it's necessary to bring more brain power to bear than any one person or laboratory team can muster.

From molecules to medicine

"THE USE OF DATA SCIENCE to understand basic biology is critical," said Sylvia Plevritis, PhD, professor of biomedical data science and of radiology and chair of Stanford Medicine's department of biomedical data science. "Without advances in data sciences, scientists can take months or years to analyze, by hand, the vast amounts of data generated by new biotechnology platforms. With these advances, we can analyze data we've never worked with before and combine data from different platforms at different scales of resolution. And we can start to resolve patterns that can direct future areas of research."

Stanford Medicine has been a leader in this area since 1982, when it launched a training program in medical information sciences — the first of its kind in the world. The program, which subsequently became the biomedical informatics program, emphasized the nexus among medicine, statistics and computer science and is now part of the Department of Biomedical Data Science, established in 2015.

Plevritis, who also heads Stanford's Center for Cancer Systems Biology, uses computers to identify drivers in the matrix of signals that control cancer cell survival. Other researchers design algorithms to predict a tumor's molecular architecture from fragments of DNA circulating in a patient's blood, or to analyze countless cross sections of tissue from thousands of patients to quickly identify people with nascent cancers.

"We are on the cusp of a time when computers are going to have a massive impact on drug discovery," said Nathanael Gray, PhD, the Krishnan-Shah Family Professor and professor of chemical and systems biology. "The challenge is figuring out how to design machine-friendly experiments, to enable the computer to understand patterns and relationships our brains can't fundamentally comprehend."

But understanding the molecular drivers of cancer or the cell structure in a suspicious growth doesn't automatically

THE EMPHASIS ON RAISING UP THE NEXT GENERATION OF STELLAR CLINICIAN RESEARCHERS REMAINS IN THE FORM OF STANFORD'S LONG-STANDING MEDICAL SCIENTIST TRAINING PROGRAM, WHICH ALLOWS STUDENTS TO SIMULTANEOUSLY OBTAIN A MEDICAL DOCTORATE AND A RESEARCH DOCTORATE IN A SIX- TO EIGHT-YEAR PERIOD OF INTENSE LEARNING.

translate to immediate changes in patient care. Sometimes, good ideas born on the lab bench or computer keyboard stumble and die in the face of the daunting amount of funding, expertise and time required to bring those ideas into the clinic.

"Most mere mortals can't put all the pieces together to go from a basic science observation with a potential disease link to finding a small molecule, characterizing it, proving its safety in animal models, and getting money to fully develop it into a drug that can be tested in clinical trials," said Gray, who also leads the small-molecule drug discovery program at the Innovative Medicines Accelerator.

The accelerator helps researchers advance basic science discoveries across translational medicine's "valley of death" — the chasm that yawns between an idea in the lab and the first test of a new drug in people. The goal is to reduce the time and cost of drug development and to deliver more effective medicines to patients by linking researchers to the technology, resources and expertise necessary to successfully bridge that gap.

A complementary program, SPARK, educates researchers and clinicians on how to work with partners in industry and academia to move projects from bench to bedside and trains students about the ins and outs of launching their own startup companies around promising drugs or discoveries.

SENIOR ASSOCIATE DEAN FOR RESEARCH RUTH O'HARA SAYS THIS IS 'AN EXTREMELY IMPORTANT POINT IN SCIENTIFIC HISTORY.'

"These programs exist to help people along that road. Old-school drug discovery was very phenotype- and organism-based because we didn't know the molecular details behind diseases. The molecular biology revolution in the mid-1900s spurred the idea of target-based — or molecule-based — therapy, but that can have problems because it is very reductionist," said Gray. "Most modern drug discovery programs work from the top down, conducting population-based genetic studies of disease, as well as from the bottom up, thinking about molecular structure and atomic interactions. At Stanford, we have people with expertise at every step of the process."

Programs like the Innovative Medicines Accelerator and SPARK will be critical components of the Life Sciences Initiative.

"Across the country, academic medical centers are focusing, from the very earliest stage of training, on molecular medicine," said O'Hara, who is also the director of the Stanford Center for Clinical and Translational Research and Education, or Spectrum. "Stanford is a preeminent basic science research institute, and this research occurs in clinical as well as across the basic science departments. We are optimally placed to translate promising discoveries into the clinical setting. The potential we're seeing in fields like cancer immunotherapy, for example, is beyond exciting. I am a cautious person, but I believe we're observing one of the most fundamental biomedical revolutions in real time."

Big ideas lead to big change. But sometimes big changes rest on tiny but mighty foundations. Look around. What do you see? **SM**

— Contact Krista Conger at kristac@stanford.edu

CRYOGENIC ELECTRON MICROSCOPY IS DRIVING A BURST OF DISCOVERIES ABOUT HOW MOLECULES WORK IN HEALTH AND DISEASE

IMAGING BLOWS UP

BY HANAE ARMITAGE

PHOTOGRAPHY BY TIMOTHY ARCHIBALD

The molecules of life — proteins, DNA and RNA — have many looks. Some are tubular or spiraled; others are long and spindly, or bulbous and squat. And they're not stagnant. Like all molecules, they are a conglomerate of smaller bits — typically, some combination of oxygen, carbon, nitrogen, phosphorous, sulfur and hydrogen atoms connected via bonds of varying strength — and they morph as they perform the many jobs upon which human biology depends.

Even when the body is perfectly still, our insides are moving.

That molecular jostle is key to how every cell and organ functions.

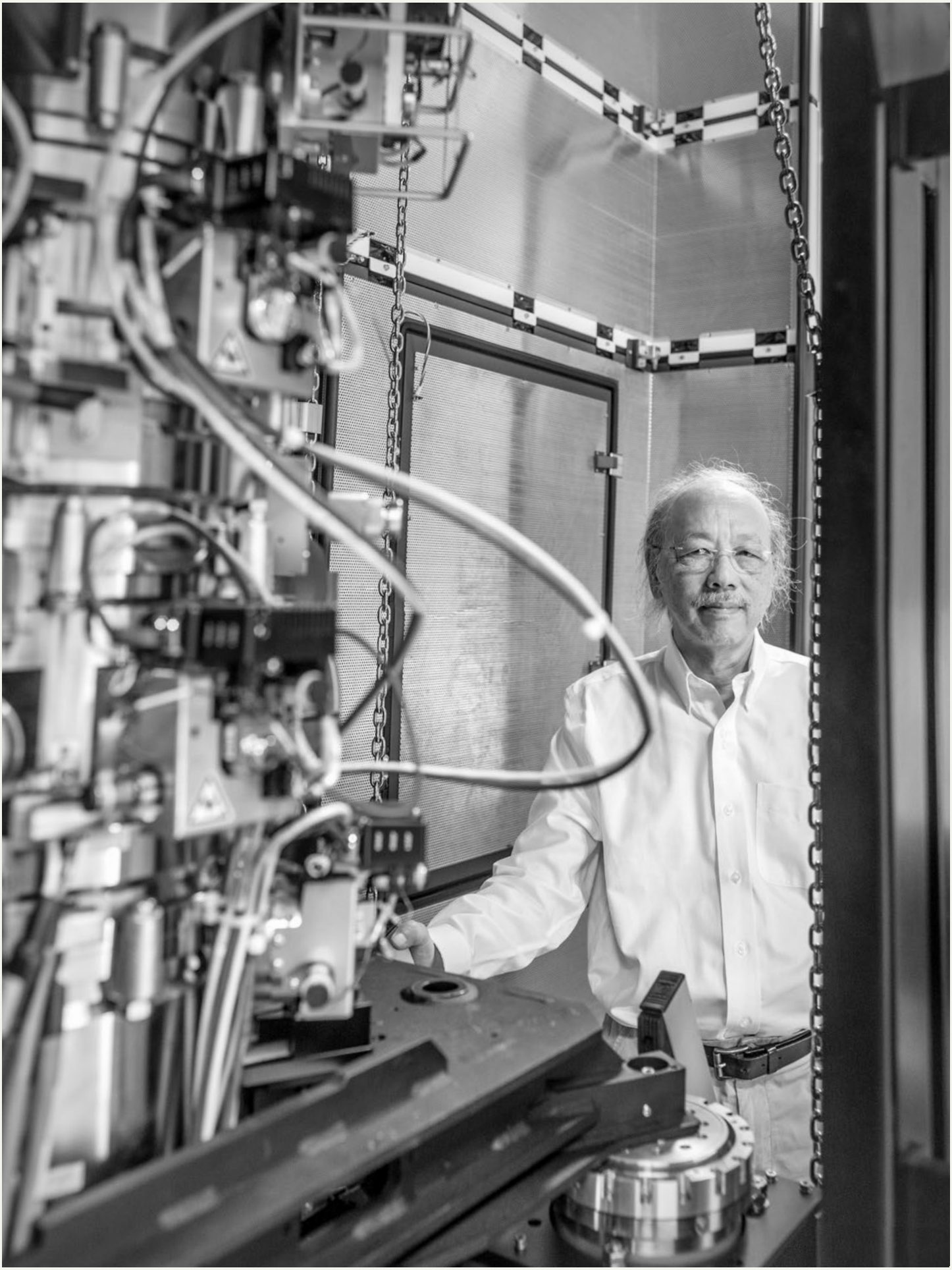
It's also a serious challenge for scientists to picture clearly.

And so was born a decadeslong quest to capture crisp images of molecules in action.

Early in the field of molecular imaging, the best depictions of molecules were akin to a blurry Rorschach test, showing the overall shape of a molecule in its crystallized state, but not much else.

Now, an imaging technique known as cryogenic electron microscopy, or cryo-EM, helps researchers decipher molecules' structures in fine detail, even as they flex, twist and undulate.

Christopher Barnes AT AN ELECTRON MICROSCOPE IN THE STANFORD CRYO-EM CENTER, WHERE HE STUDIES VIRUSES INCLUDING SARS-COV-2 AND HIV.

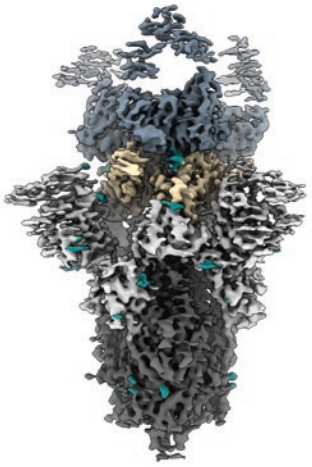


In a nutshell, the cryo-EM process comes down to roughly four steps: Freeze, shoot, detect, reconstruct. Scientists freeze a molecule of interest, pass a beam of electrons through it, and record how the electrons bounce off the molecule. That produces images of the molecule, each viewed from a different direction and each molecule caught, frozen, in a certain shape. Researchers can also combine multiple snapshots of a molecule in different conformations, creating movies of how molecules wiggle about, often in different functional states.

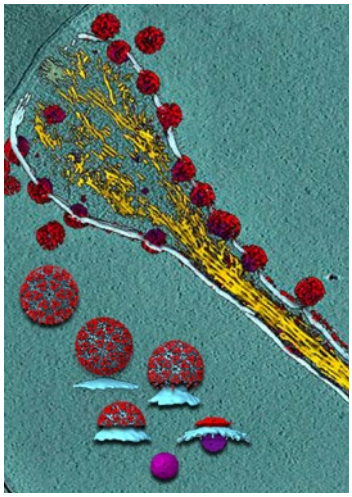
Cryo-EM holds several advantages over other molecular imaging methods: It enables investigation of a wider variety of molecules; it allows scientists to see how these molecules exist and act in their natural state or when battling a virus; and the process is relatively fast — if all goes according to plan, a scientist can freeze, shoot and create a model of a molecule in a few days. All of this adds up to a boon for designing drugs and understanding biology and disease.

Cryo-EM has been around since the 1980s, though it wasn't always the master filmmaker scientists know and love today. With developments in microscope technology and enhanced imaging resolution, it started to gain a little traction in the early 2000s, though by 2002, only eight entries were published in the Electron Microscopy Data Bank, a repository scientists use to collate data about molecular structures.

The boom in cryo-EM-based structure solving took off over the past seven years, thanks to technological advances that not only enhanced its utility as an imaging tool but also made it easier for scientists to use. In 2021 alone, 4,483 cryo-EM structures were deposited in the bank. More than 100 cryo-EM laboratories are spread across the country, and dozens more throughout the world. That may sound like plenty to go around, but for structural biologists, having an electron microscope at your institution is a big deal.



A CRYO-EM MAP OF THREE ANTIBODY FRAGMENTS (SLATE) ATTACHED TO A SARS-COV-2 VIRAL SPIKE PROTEIN. WHEN THIS TYPE OF ANTIBODY LATCHES ON TO THE VIRUS'S BINDING SITE (WHEAT), IT PREVENTS THE VIRUS FROM ATTACHING TO AND ENTERING A HUMAN CELL.



A CRYOGENIC ELECTRON TOMOGRAM OF A CELL INFECTED WITH THE CHIKUNGUNYA VIRUS DISPLAYS VIRUS (RED) ASSEMBLY AND BUDDING AT THE PLASMA MEMBRANE (LIGHT BLUE). THE CELL'S CYTOSKELETON IS DISPLAYED IN YELLOW. AT THE LOWER LEFT CORNER ARE THE VIRUS ASSEMBLY INTERMEDIATES, WITH VIRAL SPIKES IN RED, NUCLEOCAPSIDS IN PURPLE AND MEMBRANE IN LIGHT BLUE.

Today, cryo-EM is not just one of the tools for discovering a molecule's structure — it's the top tool. Three researchers who developed the technology won the 2017 Nobel Prize in Chemistry for advancing the imaging method.

"Cryo-EM is the most sophisticated technique for studying the structure of proteins," said Lloyd Minor, MD, dean of the Stanford University School of Medicine. "We made deep investments a number of years ago and essentially went from having almost no presence in cryo-electron microscopy to being an institution that leads in cryo-EM-based research."

Stanford Medicine obtained five of the multimillion-dollar microscopes operated under the Stanford Cryo-EM Center, and all Stanford faculty have access to the equipment. With these instruments in place, over the past few years the university recruited a number of researchers who excel at this type of structural biology.

The Stanford-associated SLAC National Accelerator Laboratory has an additional five cryo-EM microscopes, with two more on the way, that serve researchers from all over the country. And it's not just structural biologists who are invited to use the scopes; cryo-EM experts work with researchers both inside and outside of Stanford to help guide the use of the technology and to help them conduct projects that could benefit from a look at molecular structure.

With the influx of this machinery has come a flurry of new findings by Stanford researchers: Some paint a picture of newly identified lock-and-key binding sites that could be used to guide drug development; some fill in details about the nature of the virus behind COVID-19; and all provide insights about the molecules that make up our bodies and about the diseases that affect them.

TOP: CHRISTOPHER BARNES; BOTTOM: DAVID CHMIELEWSKI AND JING JIN

Wah Chiu STANDS ALONGSIDE AN ELECTRON MICROSCOPE LOCATED AT THE STANFORD-SLAC CRYO-EM CENTER.

"Understanding a protein's structure allows you to understand its mechanism of action; anything that affects the structure can lead to dysfunction and potentially disease," said Georgios Skiniotis, PhD, professor of molecular and cellular physiology and of structural biology at Stanford Medicine, professor of photon science at SLAC and scientific director of the Stanford Cryo-EM Center.

BEFORE CRYO, THERE WERE CRYSTALS

Cryo-EM has taken decades to reach its potential. "We used to call cryo-EM 'blobology' back in the early 2000s," said Christopher Barnes, PhD, assistant professor of biology, whose research interests include HIV and SARS-CoV-2, the virus that causes COVID-19.

In its early days, cryo-EM could help scientists understand a protein's general shape and likeness, but it couldn't show the details necessary for designing new drugs or fully understanding how the protein interacted with other molecules.

At the time, structural biologists' preferred imaging method for big biological molecules was X-ray crystallography, the same technique that Rosalind Franklin — a pioneer of structural biology — used to capture the first images of DNA's twisted-ladder likeness.

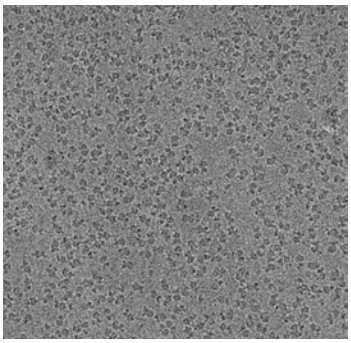
To carry out X-ray crystallography, scientists combine a supersaturated mix of a protein of interest with solutions that help the protein molecules coalesce and eventually form a crystal structure. X-ray beams are shot at the crystal, causing them to diffract, creating an interference pattern that scientists use to infer the structure of the protein.

X-ray crystallography has pitfalls, though, namely, that large molecules are reluctant to form the crystals that are crucial for imaging. Without crystals, there can be no light diffraction, no resulting pattern and no 3D image. Even if scientists could coax a protein into crystallization, there's no way to capture its dynamic nature. Instead, it's caught in a single state — like a tiny Han Solo frozen in its own version of carbonite.

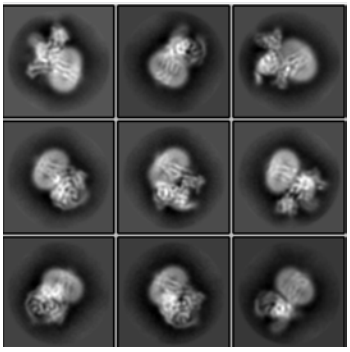
"It only gives you a snapshot of one state of the molecule," said Skiniotis.

In cryo-EM, millions of proteins swimming in a solution are dropped onto what scientists call a grid — a dish about half the size of a pencil-top eraser composed of thousands of little squares. Liquid ethane, which sits at a crisp minus 165 degrees Celsius or thereabouts, flash freezes the proteins in various conformations. The scientist then slips the icy tray under the microscope, which shoots it with a beam of electrons (without damaging the molecules), not unlike how scientists shoot X-rays at crystallized proteins during X-ray crystallography.

The electrons hit the frozen specimen and ricochet off, creating a pattern of scattered electrons, which an electron detector captures and turns into an image. Each image is a density map that reflects where the highest concentration of molecular material is located (much in the same way a population density map reflects where the most people live).



A RAW CRYO-EM IMAGE OF THOUSANDS OF THE SAME MOLECULE. A BEAM OF ELECTRONS ALLOWS THEIR PROJECTIONS TO BE RECORDED.



THOUSANDS OF PROJECTIONS ARE GROUPED BY THEIR ORIENTATION, MAKING THEM MORE VISIBLE.

A few thousand images from different areas of the grid are collected over several hours, each image typically containing two-dimensional maps, called projections, of tens to hundreds of copies of the molecule of interest. Scientists then use computer software to process the data and reconstruct the three-dimensional shape and structure of the protein. The images are snapshots of copies of a protein, which may be in different conformations, allowing researchers to create movies of protein motion in 3D.

Cryo-EM went through something of a revolution in 2013, when a new era in precise electron detectors, along with leaps in computational power, brought finesse and efficiency to the technique. Armed with new images that reveal a trove of structural information, the science of molecular biology entered its own revolution.

Just as automatic transmissions made cars more accessible to new drivers, advances in detectors and data analysis have expanded access to cryo-EM for structural biologists — and other scientists too.

GEORGIOS SKINIOTIS

Georgios Skiniotis, right, IS THE SCIENTIFIC DIRECTOR OF THE STANFORD CRYO-EM CENTER.


eppendorf

"You don't need to know the physics of a microwave to use a microwave," said Wah Chiu, PhD, a professor of microbiology and immunology and of bioengineering and the director of the Cryo-EM and Bioimaging Division at SLAC. "That's what it's like for cryo-EM now. Suddenly, people are solving structures right and left."

Stanford's leading cryo-EM researchers are using the technique to develop viral vaccines, devise new viral antidotes and even concoct new treatments for physical and psychic pain.

KNOW THY ENEMY

The quest for an HIV vaccine has been underway for decades; but despite intensive research and clinical trials, no HIV vaccine has been approved in the United States. Though treatment options for HIV infection have improved over the past few decades and fewer people die of it, the medicines still have side effects and can be cost-prohibitive, and access is still limited in some countries.

That's not to say there hasn't been progress. Recently, researchers in academia and industry have launched trials exploring an mRNA-based vaccine for HIV.

Barnes, a structural biologist recruited for his expertise in cryo-EM, is focusing his research on the envelope glycoprotein, a protein complex that decorates the surface of the virus and plays a crucial role in HIV's initial infection of cells.

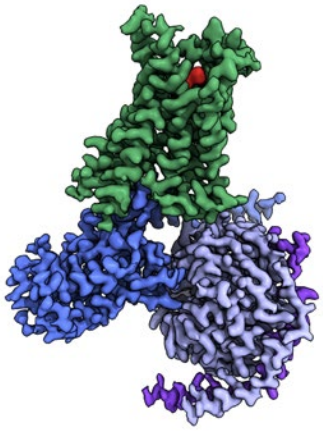
The immune system recognizes that protein complex as an unwanted invader. When the body is infected with HIV, antibodies latch onto the complex and send a signal to the immune system: This molecule is foreign and dangerous; attack. Barnes is using cryo-EM to identify the structures of HIV's pernicious envelope glycoprotein and how antibodies bind to them.

"That's really important for helping us understand the most potent mechanism to inhibit viral infection," said Barnes. In particular, he's interested in one key piece of the protein complex: the region where antibodies bind, known as the epitope.

But there's more than one type of epitope onto which the antibodies latch, and some epitopes are more conserved than others among the nine existing subtypes of HIV. "Structural biology can help us identify and characterize those highly conserved regions, which we want to use to design vaccines," said Barnes. "At the end of the day, you want a vaccine that's going to induce antibodies that target the most conserved binding regions across HIV strains."

In past work, Barnes identified antibodies that target an envelope region that's shared across 70% of HIV varieties, dubbed the silent face — a region that sits across from where the virus attaches itself to human cells in the early stages of infection.

"Now we want to use the structural information we have detailing that region to engineer mimics of the envelope protein to basically elicit a response from the human immune system, such as the generation of broadly neutralizing antibodies, in the hopes of stopping a real infection," said Barnes. He hopes that capturing the nooks and crannies of the epitopes in more detail than ever before will finally yield a vaccine that can snuff out all strains of HIV.



A HIGH-RESOLUTION CRYO-EM MAP OF A SEROTONIN RECEPTOR (GREEN) BINDING WITH A HALLUCINOGEN-LIKE MOLECULE (RED) AND THE THREE-PART G PROTEIN (BLUE, LILAC AND PURPLE).

GEORGIOS SKINIOTIS

Barnes is also zeroing in on coronaviruses, including SARS-CoV-2 and other animal-borne coronaviruses that have the potential to spill over into humans. "We're trying to identify antibodies that recognize entire families of coronavirus that can have broad disease-fighting capabilities," he said.

Early in the pandemic, when many people infected with SARS-CoV-2 were donating convalescent plasma for research purposes, Barnes obtained samples to isolate antibodies triggered by the virus. The goal was to use the structural properties of these protective

IT'S STILL EARLY DAYS IN CHIU'S EXPLORATION, BUT LEARNING MORE ABOUT THE STRUCTURAL DIFFERENCES DURING INFECTION AND REPLICATION — OF KEY PROTEINS OR THE VIRUS OVERALL — CAN SHED LIGHT ON HOW A VIRUS GROWS AND OVERTAKES A HEALTHY CELL.

IN DRUG DESIGN, THE MORE SPECIFIC ATOMIC-LEVEL INFORMATION AND THE MORE CONFORMATIONS CRYO-EM IMAGES REVEAL ABOUT A MOLECULE'S STRUCTURE, THE BETTER. THAT'S WHAT IMPROVES DRUG TARGETING CAPABILITIES.

antibodies to inform how to create possible treatments.

He and colleagues at Rockefeller University identified three neutralizing antibodies with the potential to wipe out COVID-19 infection, two of which are being tested as a treatment. "It was really rewarding to see this work translate to clinical studies that could help to improve outcomes for patients," Barnes said.

Chiu has also applied his structural expertise to coronaviruses, specifically the infamous spike protein — a name that conjures images of a ball decorated with sturdy spikes that pierce or jab tender cells as it infects.

Though they look sharp, cryo-EM imagery has shown what look like spikes aren't that at all. Instead, they bend, twist, shimmy, shake and jiggle for an unknown purpose. It's a mystery how mobility aids the virus's ability to infect, but the more scientists know about this key protein, especially the unexpected bits (like the bending of the spikes), the better they can understand — and hopefully stop — coronaviruses overall.

Chiu, a pioneer of cryo-EM who's approaching 50 years of experience using and improving structural biology imaging technology, is also taking a hard look at the mosquito-borne virus that causes chikungunya by zooming out to encompass a whole system of proteins and how they interact with healthy cells to infect humans.

"We're able to use cryo-EM to create images of a cell that's mid-infection with chikungunya," said Chiu. Instead of looking at a single protein of interest, Chiu's team is harnessing the movie-magic of cryo-EM to watch the chikungunya virus mature and develop inside a cell, and then burst out. It's still early days in Chiu's exploration, but learning more about the structural differences during infection and replication — of key proteins or the virus overall — can shed light on how a virus grows and overtakes a healthy cell.

He and a research team have drawn on structural biology clues to better elucidate how antibodies stop the chikungunya virus from infecting other cells: Antibodies bind to the virus and trigger a cascade of events that stop the virus from multiplying inside the cell, preventing it from exiting the cell to infect others.

THE STRUCTURE OF DRUG DESIGN

From behind round-framed glasses, Skiniotis exudes a soft-spoken, animated passion for cryo-EM, his extensive knowledge particularly on display when he talks about his investigation into G-protein-coupled receptors, a family of some of the most diverse and dynamic proteins in the human body. His drug research centers on these receptors, a class of proteins known for their role in relaying critical signals between cells and tissues to regulate myriad processes, from our heartbeat and mood to our ability to see in a dimly lit room. These receptors, located on the surface of cells, are known to be the most "druggable" class of receptors — more than 30% of drugs approved by the Food and Drug Administration target these proteins. But without an understanding of how they contort and connect with neighboring molecules to do their jobs, their drug target potential cannot be fully realized.

"One might take a snapshot of certain proteins coming together and say, 'I want to break that interaction because it leads to a certain disease. Therefore, I'm going to design a molecule that blocks a central binding site,'" said Skiniotis.

But perhaps blocking that site also affects the binding of other proteins that are beneficial to health, or perhaps it turns out that the drug you designed binds to other off-target proteins. In drug design, the more specific atomic-level information and the more conformations cryo-EM images reveal about a molecule's structure, the better. That's what improves drug targeting capabilities, he said.

"We have focused on this area for the past few years," he said. "In my book, you don't want to get into drug design if you aren't first focused on mechanisms — drug design requires an understanding of how proteins work and how this function can be blocked, enhanced or modified by certain compounds."

CONTINUES ON PAGE 55

MY FAVORITE MOLECULE

A dozen Stanford Medicine researchers explain what piques their interest when it comes to molecules

For kids on a playground, it's a common refrain between new friends:
"What's your favorite color?
What's your favorite animal?"
For young adults, they make good icebreakers on dates:
"What's your favorite place in the world?
What's your favorite pizza topping?"
A person's favorites often cut to the punch of their personality, likes and preferences —
although, admittedly, a favorite book or hobby might say more about someone than their favorite dinosaur.
In the professional world, favorites rarely come up, but surely scientists have them,
whether they're favorite conferences, journals, papers or experiments.

FOR THIS ISSUE,
we asked 12 Stanford Medicine researchers to tell us about their favorite molecules.
It turned out to be a good way to give insight into their research:
Every scientist — perhaps unsurprisingly — named a molecule that they actively study.
In most cases, the potential impact of a molecule on human health topped the list of reasons a researcher calls it a favorite.
But for some scientists,
they consider a molecule their favorite because it is understudied, has multiple roles in the body,
has a neat three-dimensional structure or has been key to their professional success.

BY SARAH C.P. WILLIAMS

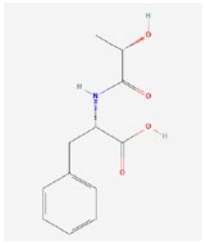
ILLUSTRATION BY JEFFREY DECOSTER

Jonathan Long, PhD

ASSISTANT PROFESSOR OF PATHOLOGY

LAC-PHE

If you've ever felt queasy after a long run or lost your appetite for breakfast after a morning spin session, then you have firsthand experience with Jonathan Long's favorite molecule: a chemical known as Lac-Phe.



Long's lab group recently discovered that this tiny signaling molecule shuts down hunger signals in the brain after strenuous exercise. When you exercise, lactic acid builds up in your muscles, creating several bioactive byproducts, one of which is Lac-Phe. Long's team members think that during and after a workout session, the molecule races from the muscles to the brain and acts on neurons to turn down the dial on hunger, though the researchers haven't pinpointed just how this happens.

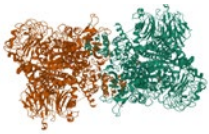
"What's neat is that Lac-Phe is present in every animal that moves, meaning it's extremely conserved and ancient," said Long. "Understanding it better might help us capture the benefits of exercise to treat all kinds of things, from osteoporosis to obesity."

Suzanne Pfeffer, PhD

PROFESSOR OF BIOCHEMISTRY AND THE EMMA PFEIFFER MERNER PROFESSOR IN MEDICAL SCIENCES

LRRK2

When Suzanne Pfeffer learned of LRRK2, she was immediately captivated; LRRK2 is the most commonly mutated protein in inherited Parkinson's disease, but researchers didn't understand why.



During the past five years, Pfeffer's lab group has pieced together much of the mystery, revealing how mutations in LRRK2 make a small set of brain cells lose their primary cilia — miniscule, molecular antennae that nearly every human cell uses to communicate. Without primary cilia, the cluster of neurons in the brain can't receive vital stress signals sent by neighboring cells, so they don't send back necessary protective factors. The sets of neurons on both sides of the interaction begin to die.

Some pharmaceutical companies are testing drugs that quell the activity of LRRK2 in patients with Parkinson's disease. But Pfeffer isn't moving on from her favorite molecule yet; she thinks it has many important secrets that are yet to be discovered.

"We still don't know why this one group of cells in the brain is so uniquely sensitive to LRRK2 mutations, considering LRRK2 is found throughout the rest of the brain and body too," she said.

Roger Kornberg, PhD

MRS. GEORGE A. WINZER PROFESSOR IN MEDICINE

THE NUCLEOSOME

A strand of DNA, yanked out of a cell, is roughly as long as an average adult is tall. In 1974, Roger Kornberg discovered how nucleosomes — clusters of proteins bound to DNA like beads along a string — package this lanky genetic material into tightly folded structures inside cells.

"The nucleosome was probably the most important discovery of my career," said Kornberg, whose career includes a Nobel Prize in Chemistry. "Working alone, as a very young scientist, I conceived of the solution of this DNA packaging problem that had been studied for decades."

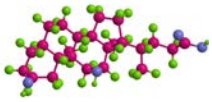
In the years since, Kornberg and his wife and longtime collaborator, Yahli Lorch, PhD, have revealed how nucleosomes are more than just physical spools; the molecular complexes control which genes are expressed in which cells in the body. Genetic material wound around nucleosomes can't be accessed by the cellular machinery that reads DNA, so the packaging of nucleosomes determines which genes a cell is using.

Michael Fischbach, PhD

ASSOCIATE PROFESSOR OF BIOENGINEERING AND OF MEDICINE

DEOXYCHOLIC ACID

For the past five years, Michael Fischbach has been enamored of a molecule that, he said, "basically makes up the exhaust fumes of bacteria in the gut." Our intestines, however, put this bacterial waste — deoxycholic acid — to good use, using it to absorb fats in our intestines.



"I'm fascinated by this fact that there are hundreds of molecules in circulation in our bodies that weren't made by us but impact our biology," said Fischbach.

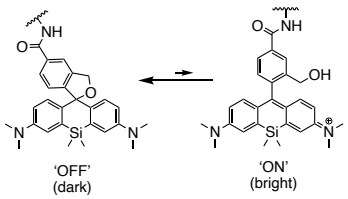
Deoxycholic acid not only digests fatty foods better than cholic acid — the version of the molecule that our own bodies make — but also binds to receptors in the intestines, having far-reaching effects on metabolism and immunity.

Jonathan Tyson, PhD

POSTDOCTORAL SCHOLAR IN BIOENGINEERING

HYDROXYMETHYL SILICON RHODAMINE

Trying to peer inside a living cell and keep track of all the components is like trying to hear a conversation in a noisy cafeteria, said Jonathan Tyson. Everything blurs together. The fluorescent molecule hydroxymethyl silicon rhodamine, or HMSiR, changes that; unlike most fluorescent tags that remain steadily on, it blinks on and off, illuminating at any given time just a small percentage of the molecules that it is attached to. "HMSiR makes every molecule speak one at a time, with decorum," said Tyson. "Suddenly you can understand what they're all saying."



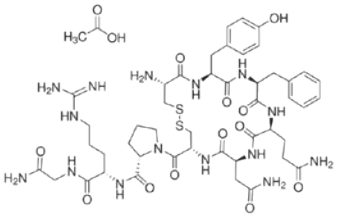
Tyson relied on HMSiR to carry out his graduate work, revealing the minute details of cellular organelles and their movements and shape-shifting over long periods of time. He also designed other, similar molecules from scratch to allow more precise, nano-scale microscopy. Now, he's studying how related "blinking" molecules might be useful in building molecular devices on and in cells rather than simply illuminating cellular components.

Karen Parker, PhD

ASSOCIATE PROFESSOR OF PSYCHIATRY AND BEHAVIORAL SCIENCES

VASOPRESSIN

When it comes to molecules, Karen Parker has a soft spot for the underdog. "For many years, vasopressin has been in the shadow of oxytocin, which has gotten all of the scientific attention," she said. "That's part of why I like it so much; it's really under-researched."



Oxytocin is often called "the love hormone" because its activity in the brain is associated with attachment, trust and social interaction. But Parker thinks that vasopressin is just as important; she discovered that people with social impairment disorders have lower than usual amounts of vasopressin — but not oxytocin — in their spinal fluid. She and her colleagues can even predict which newborns will develop autism based on these vasopressin levels, and she has shown that giving vasopressin to children with autism boosts their social abilities and diminishes anxiety.

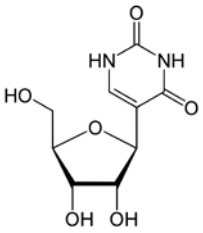
"Since we've been publishing papers on vasopressin, I've seen an uptick in interest," said Parker. "I hope it continues to get more love."

Nicole Martinez, PhD

ASSISTANT PROFESSOR
OF CHEMICAL AND SYSTEMS BIOLOGY AND OF
DEVELOPMENTAL BIOLOGY

PSEUDOURIDINE

Nicole Martinez's favorite molecule is like a code within a code. Imagine you were reading an instruction manual and every time you saw a backwards R, you'd have to pull the manual apart, assemble it in a new way and follow new instructions. Those Яs are kind of like pseudouridine — a modified, rotated version of the RNA building block called uridine.



When an RNA molecule contains pseudouridine in place of uridine, it can change how the molecule is processed, how it functions, how stable it is and even whether the immune system can recognize it as RNA. But researchers are stymied by the details on how and why; Martinez is trying to break the code.

"I'm just fascinated by this idea that in addition to the basic sequence of RNA, there's an additional layer that can change the meaning of RNA's message," said Martinez.

Alka Das, PhD

POSTDOCTORAL SCHOLAR IN
MOLECULAR AND CELLULAR PHYSIOLOGY

MEC-4

In the *Caenorhabditis elegans* roundworm used frequently in biology labs, the protein MEC-4 acts like a tiny tripwire. The doughnut-shaped MEC-4 spans the membrane of some *C. elegans* cells. When a predator pokes, prods or squeezes the worm, MEC-4 senses the pressure and opens its central channel, letting charged molecules flow through.

"When worms don't have MEC-4, they actually can't sense any gentle touch," said Alka Das, who studies the protein. "It's really fascinating to me that these proteins can translate a physical force into a biological response."

While scientists have characterized other touch-sensitive ion channels, MEC-4 has a drastically different sequence and is finicky in the lab, Das said. She is still trying to determine its three-dimensional structure as well as what other proteins it interacts with.

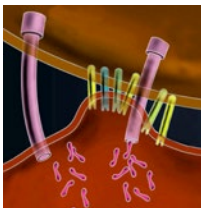
Manuel Amieva, MD, PhD

PROFESSOR OF PEDIATRICS
AND OF MICROBIOLOGY AND IMMUNOLOGY

CAG BACTERIAL TYPE IV SECRETION SYSTEM

When *Helicobacter pylori* bacteria slide down someone's esophagus into the stomach (and they do this often, having infected about half the world's population), they are armed for trouble. The microbes are coated with miniscule needles — called the bacterial type IV secretion system — that poke into human cells to inject proteins.

"I find it amazing that bacteria evolved to have these tricky little nano-syringes," said Manuel Amieva.



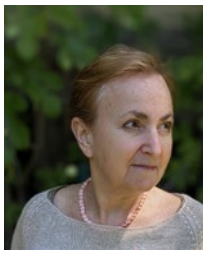
In *H. pylori*, the type IV secretion system is quite complex, with about a dozen different protein subunits, but it has just one known job: delivering a protein known as CagA, which can boost people's risk of ulcers and stomach cancer. For the past two decades, Amieva has studied CagA — how it can contribute to cancer risk and why *H. pylori* has the protein in the first place.

The secretion system and its payload, which Amieva ranks as his favorite molecular structure, are an example of how bacteria have evolved sophisticated communications systems with their host, he said.

Daria Mochly-Rosen, PhD

PROFESSOR OF CHEMICAL AND SYSTEMS BIOLOGY AND THE GEORGE D. SMITH PROFESSOR IN TRANSLATIONAL MEDICINE

DRP1



As Daria Mochly-Rosen describes her favorite protein, Drp1, her arms rise in front of her body, elbows swinging one way and then another to show how the hinged protein moves.

"I'm a protein chemist and I just love to look at how proteins move," she said. "Drp1 is a fun little machine."

Inside cells, Drp1 molecules arrange themselves, arm over arm, around mitochondria — organelles that, among other things, generate energy. When the Drp1 ring tightens, it pinches a mitochondrion in two. But Drp1 has different partners for this dance. When it binds one protein, mitochondrial division progresses at a normal pace; when it binds another, mitochondria divide too much and too frequently — potentially contributing to neurodegeneration.

Mochy-Rosen's lab has developed a drug that keeps Drp1 from binding to the more toxic dance partner while still allowing its normal function. "If we learn how to control Drp1, we can improve mitochondrial function, which is essential to treating many pathologies including neurodegenerative diseases," she said.

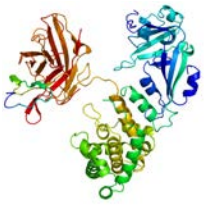
Peter Santa Maria, MD, PhD

ASSOCIATE PROFESSOR OF OTOLARYNGOLOGY - HEAD AND NECK SURGERY

HEPARIN-BINDING EGF-LIKE GROWTH FACTOR

Peter Santa Maria's molecule of choice is an earful, both literally and figuratively. His lab discovered in 2014 that heparin-binding EGF-like growth factor, or HB-EGF for short, can regenerate a damaged eardrum. While some people's eardrums manage to repair themselves after a rupture, others never heal on their own. These unhealed ruptures — especially common in developing nations — can cause hearing loss and recurrent ear problems.

Santa Maria's lab showed that, in mice, HB-EGF can coax chronic eardrum perforations to close. The pharmaceutical company Astrellas Pharma Inc. licensed the therapy, from a startup that Santa Maria co-founded, and is now enrolling patients in an early-phase study to test how well HB-EGF works in humans with chronically ruptured eardrums.



"This is the molecule that I've had the most joy with," said Santa Maria. "If it keeps performing well in clinical trials, I think it will be my favorite for life."

Kathleen Ruppel, MD, PhD

SENIOR SCIENTIST IN BIOCHEMISTRY

MYOSIN

In 1987, Kathleen Ruppel was a first-year MD/PhD student sitting in a Stanford Medicine lecture hall when biochemistry and structural biology professor James Spudich, PhD, showed the class a video of *his* favorite molecule as it moved fluorescent filaments across a microscope slide. It was myosin — a tiny motor that powers movements within cells.

"I was hooked on myosin pretty much right away," said Ruppel.

Myosin has remained Ruppel's favorite molecule, and — now a pediatric cardiologist — she has teamed up with Spudich to study its array of functions in the human body, especially the heart. Versions of myosin not only power muscles, from biceps to the beating heart, but also play roles in the smaller-scale movements of materials in and out of cells.

Ruppel said the fact that each type of myosin molecule has its own quirk keeps the research interesting; some walk hand over hand like orangutans hanging from a branch while others stroke in synchrony like oars in a boat. **SM**

— Contact Sarah C.P. Williams at medmag@stanford.edu

VICIOUS CIRCLES

CANCER'S DEADLY WEAPON— RINGS OF DNA— HAVE BEEN HIDING IN PLAIN SIGHT

BY KRISTA CONGER

PHOTOGRAPH BY TIMOTHY ARCHIBALD

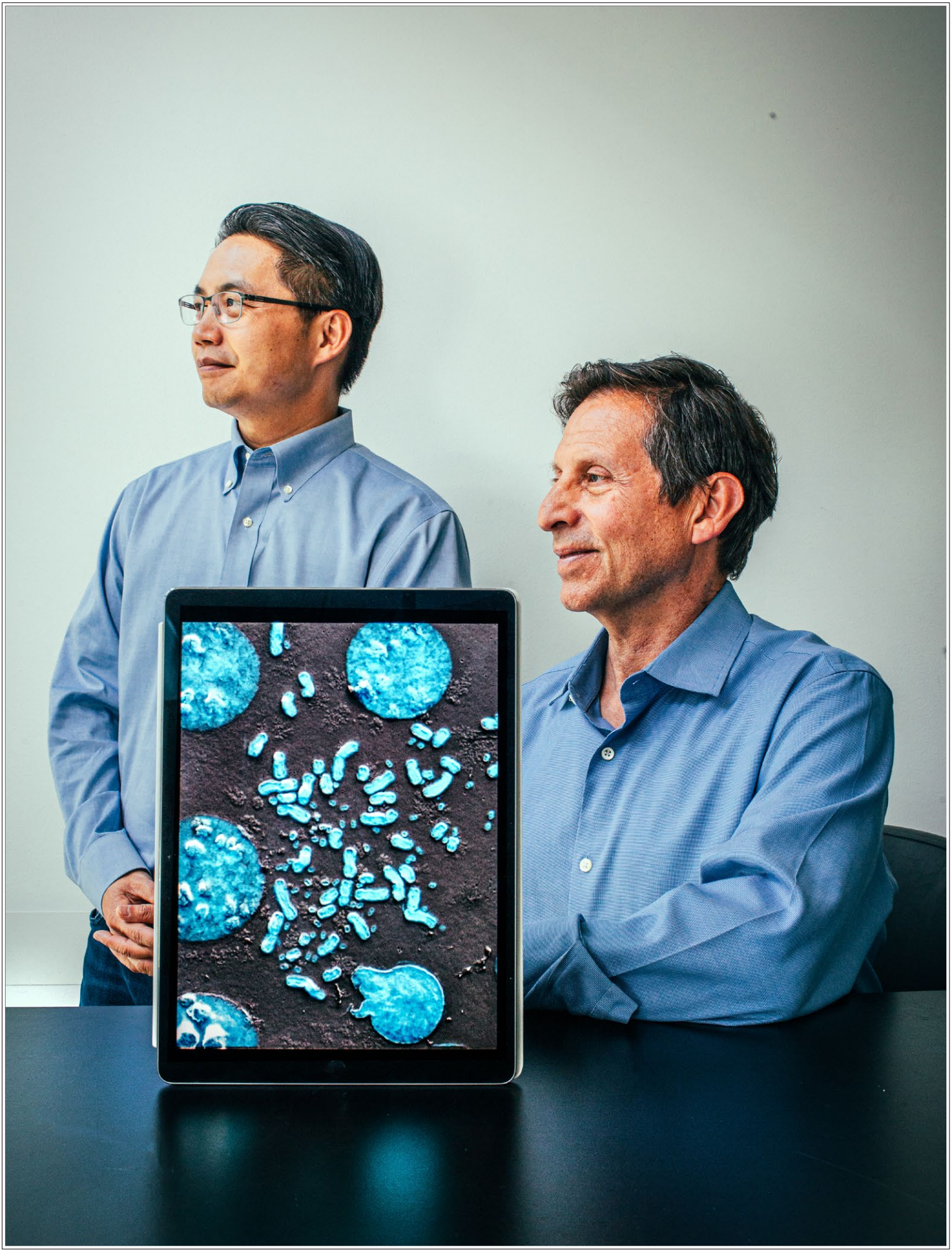
LOCATION, LOCATION, LOCATION.
IT'S NOT JUST IMPORTANT IN REAL ESTATE BUT ALSO IN BIOLOGY AND, APPARENTLY, RESEARCH SEMINARS.

It was a Tuesday afternoon in December 2017, and Paul Mischel, MD, a cancer biologist at UC San Diego, had just finished giving a talk at Stanford describing a surprising observation: small circles of DNA in cancer cells bobbing in the cells' nuclei, untethered to nearby chromosomes — the multiple long chains of DNA that comprise the cells' genetic material.

The circles, known as extrachromosomal DNA, or ecDNA, had been dismissed for decades by mainstream geneticists as a biological fluke. But a few years earlier, Mischel had begun to suspect there was more to the free-floating, SpaghettiOs-shaped structures.

His hunch was right. We now know that the circles, which are only occasionally found in healthy cells, are chockablock with cancer-causing genes. They are a primary driver in cancer growth and the evolution that helps some tumors evade drug therapies within weeks or months. Unfortunately, they are

HOWARD CHANG, LEFT, AND PAUL MISCHEL ARE TURNING CANCER RESEARCH ON ITS HEAD WITH THEIR DISCOVERIES ABOUT RINGS OF DNA THAT EXIST OUTSIDE OF CHROMOSOMES. HERE, THEY DISPLAY A RENDERING OF THE RINGS (SMALLEST CIRCLES) MIXED WITH CHROMOSOMES (OBLONGS) AND NUCLEI (LARGE SPOTS).



not rare: 1 in 3 cancer patients, often those with the most aggressive types of cancer, have high levels of ecDNA in their tumor cells.

Recently, the circles' importance has been internationally recognized. In 2021, the National Cancer Institute and Cancer Research UK partnered to select ecDNA as one of eight Cancer Grand Challenges with the potential to advance cancer research and improve the lives of people with cancer. And in June, Mischel and his team were selected from a panel of global applicants to receive $25 million from the partnership to continue their research into ecDNA in cancer.

"We now have an unparalleled opportunity to move from incremental research advances to transformational science," Mischel said. "Patients whose cancer cells have lots of ecDNA fare much more poorly than their peers do. There is a massive medical need to understand how they function."

But how do you get to the bottom of a circle?

At the time of the 2017 seminar, only a few researchers were exploring the role of ecDNA in cancer. But Mischel's audience, including Howard Chang, MD, PhD, a professor of genetics and Stanford Medicine's Virginia and D.K. Ludwig Professor in Cancer Research, was intrigued. Chang was studying when and how genes are turned on, or expressed, in cancerous and healthy cells.

"After the talk, Howard came up and said, 'Hey Paul, I think we might be seeing something similar in our data,'" Mischel recalled. "It was really a life-changing moment for me."

Mischel, who joined Stanford Medicine in 2021 as a professor of pathology, and Chang decided that day to team up to learn more about ecDNA and how it functions in cancer patients. Their results turned traditional genetics on its head and spawned an entirely new field of research.

"The ways in which these circles interact to affect gene expression to drive cancer growth is an entirely new concept in molecular biology," Chang said. "We believe it will rewrite biology textbooks."

Chang describes the circles as vicious gangs that terrorize the chromosome-bound genome by ignoring all the understood rules of biology, making cancer therapies for some patients a game of whack-a-mole as tumors evolve drug resistance within days or weeks.

In short, they are agents of chaos. And stopping them has become a primary goal of cancer researchers worldwide.

NOW WAIT A GOSH DARN MINUTE

Mischel's talk wasn't the first time ecDNA had been described in cancers. Microbiologists in the mid-1960s who observed the ring-shaped structures near chromosomes called them "minutes" (with a long vowel i), meaning tiny. Two circles linked together in a figure eight structure were called "double minutes."

Having named them, but without having the tools to study them in greater detail, biologists for the most part ignored them. Instead, genome biologists focused on mapping the locations of and, later, sequencing individual genes on each of the 23 pairs of chromosomes in each mammalian cell.

Chromosomes are made up of genes and regulatory regions — switches that determine when and where the genes turn on and off — linked arm in arm like the setup for the childhood game of Red Rover. To fit inside the cramped space of the nucleus, the strand of tens of millions of genetic building blocks twists tightly around itself and winds around packaging proteins called histones, like a line of excited hand-holding kindergartners crowding around a puppy.

Until about 50 years ago, geneticists and biologists believed that mammalian cells had two, and only two, copies

'WE BELIEVE IT WILL REWRITE BIOLOGY TEXTBOOKS.'

of each gene — one on each member of the chromosomal pair (with the exception of genes found on the sex chromosomes, which differ in their gene makeup). They also believed chromosomes were aloof: Regulatory regions on one chromosome didn't affect genes on another.

In the 1970s, however, the late Stanford biologist Robert Schimke, MD, and his lab performed a series of experiments that showed that mammalian cells could in fact harbor more than two copies of certain genes — a concept termed gene amplification. Importantly, the number of copies of an amplified gene in each cell correlated with the number of minutes or double minutes it had, and the presence of the circles was a key factor in the cells' ability to rapidly evolve resistance to a common chemotherapy drug.

Although gene amplification in mammalian cells was eventually accepted, Schimke's first public discussion of the possibility was met with skepticism. He described a session at a Cold Spring Harbor Laboratory symposium in June 1977 in which he broached the possibility as "memorable and stormy."

RISE OF THE CIRCLES

SCHIMKE'S DISCOVERY OF GENE AMPLIFICATION OPENED A NEW ERA in cancer biology. But despite his identification of ecDNA as one way cells could accumulate more than two copies of a gene, most research focused on a subsequent discovery that gene amplification could also occur on the chromosomes themselves. Curiosity about the circles subsided once again.

One reason for this is that the DNA sequencing technologies first launched in the late 1970s had no way of differentiating DNA on chromosomes from DNA in ecDNA. Also, it was believed (wrongly, it turns out) that ecDNA was rare — occurring in less than 2% of cancers.

But in 2017, Mischel and his colleagues showed that ecDNA is widespread and likely to play a major role in many human cancers.

The roots of the discovery arose from research Mischel and a colleague at UC San Diego were conducting on tumor cells from patients with glioblastoma — a highly aggressive form of brain cancer. Patients with the condition have an amplification of a gene called epidermal growth factor receptor, or EGFR, that encourages cancer cells to divide uncontrollably. At first, the researchers assumed that the extra copies of the gene resided on chromosome 7, near the original gene.

A drug targeting the EGFR protein could be expected to kill the cancer cells and slow tumor growth. But something unexpected happened. Instead of slowing tumor growth, the drug caused the cancer cells to quickly lose extra copies of the gene — much more quickly, in fact, than could be explained with traditional genetics.

Furthermore, when the researchers grew the patients' cancer cells in the laboratory, each original cell from the tumor gave rise to a colony of cells that included cells with high, low or no copies of the EGFR gene, regardless of the status of the founding cell. This was also unexpected in the chromosomal-centric view; usually a cell carrying just a few of these genes would give rise only to cells that similarly carry just a few.

"To understand what was going on, we looked inside the nucleus of the cells and got a shock," Mischel said. "We found that multiple copies of the EGFR gene were located on the ecDNA, rather than on the cells' chromosomes."

Unlike chromosomes, which are equitably distributed between daughter cells during cell division, ecDNA swirls unpredictably in the nucleus like bubbles in a bathtub and are portioned out willy-nilly to the daughters. As a result, changes in the overall genetic makeup of the tumor can happen very quickly. Within one or two generations a tumor includes cells that have many, few or none of the circles.

Imagine if each of Darwin's finches could quickly hatch hundreds of offspring with a nearly infinite variety of beak shapes, without the need to laboriously accumulate random, potentially beneficial mutations along their chromosomes. Now toss this plethora of progeny a nutritious but hard-to-crack peanut, or create a trap that ensnares only the stubby-beaked siblings. No one approach will benefit or harm all flock members simultaneously — with each challenge, some will die and others will thrive.

This is what cancer doctors and drug designers are up against when treating patients whose cancers have high levels of ecDNA, Mischel's research showed. Like the fanciful finch flock, many aggressive cancers can quickly become resistant to drug therapies targeting cells with high levels of cancer-associated proteins — they simply let those cells die and pivot to others already waiting in the wings that aren't targeted by the drug.

It's a bit diabolical.

"Darwin taught us that genetic variation is the fuel for natural selection," Mischel said. "What we were seeing was cancer evolution on steroids. It's a whole different level."

Mischel and his colleagues published their groundbreaking results in *Science* in 2014, but they were met with "a colossal scratching of heads" and not a little skepticism.

CONTINUES ON PAGE 55

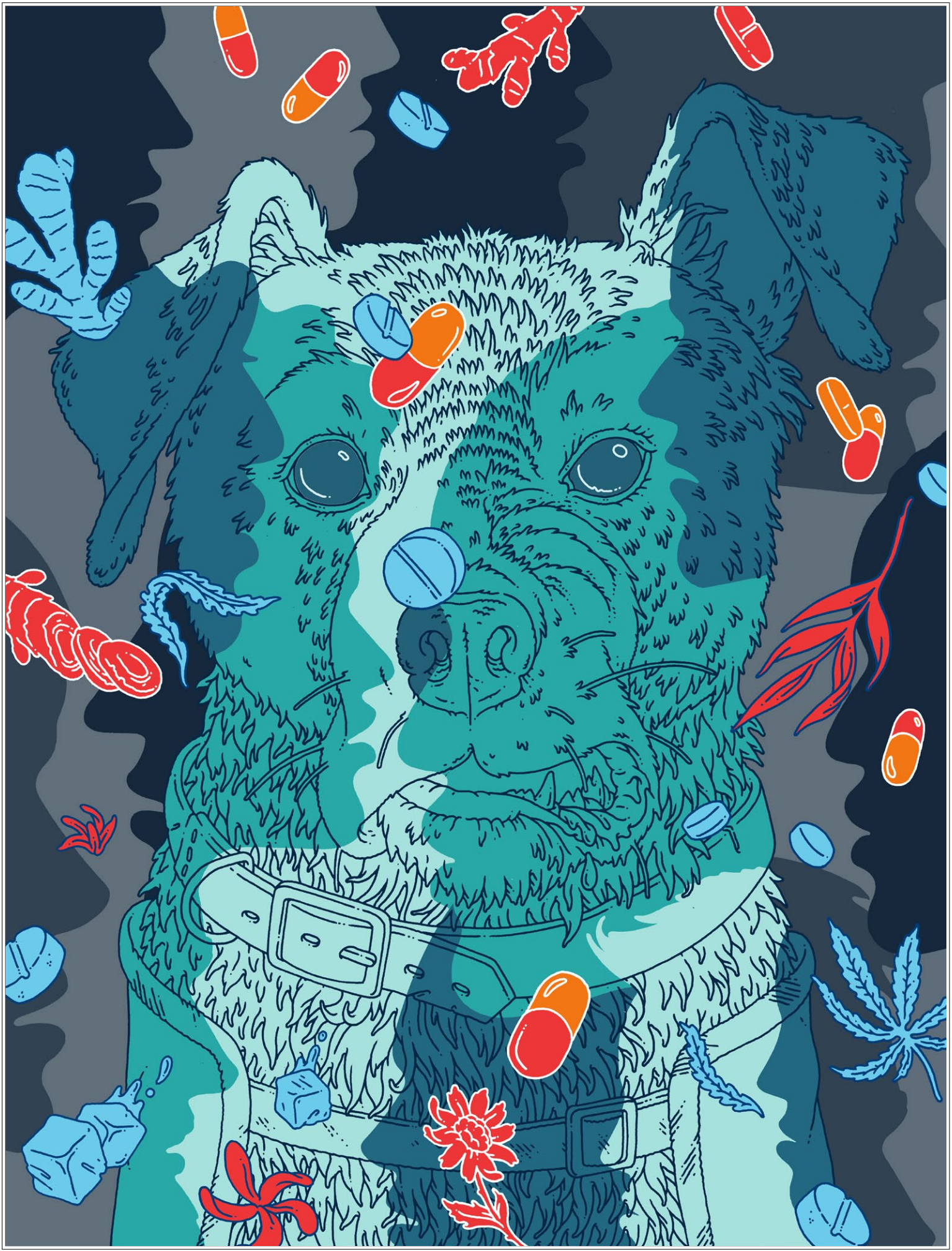
REFORMING PAIN

GOODBYE TO ONE-SIZE-FITS-ALL SOLUTIONS TO REIN IN PAIN

BY GORDY SLACK
ILLUSTRATIONS BY ERIC NYQUIST

The paradox of pain is that "it is so good, precisely because it is so terrible," said Sean Mackey, MD, PhD, who leads Stanford's division of pain medicine. Any less terrible and we'd ignore it — potentially to our mortal detriment. Healthy pain is like a service dog guiding us through a world full of perils.

For a growing number of Americans, however, that faithful dog has gone rabid and broken from its leash. According to a 2018 *Morbidity and Mortality Weekly Report* paper, co-authored by Mackey, about 1 in 5 U.S. adults suffer from chronic pain, with an even higher prevalence among women, people living in poverty, rural residents, older people and people with public health insurance. A World Health Organization study found a four-times-higher incidence of depression or anxiety among people living with chronic pain, which often interferes with the ability to concentrate, eat and sleep. And the effects of that pain on quality of life — for the primary sufferers but also the people around them — radiate out, touching nearly everyone, said Mackey. The economic impact of chronic pain is also astounding, resulting in $560 billion to $635 billion in direct medical costs and lost productivity, according to the *MMWR* paper.



Traditional treatments don't work for many sufferers, said Mackey, and they can have negative consequences; the most obvious example of treatments gone awry is the misuse of the mightiest and most notorious class of painkiller, opioids, which has fed a devastating nationwide epidemic of addiction and overdoses.

In part a corrective reaction to the one-size-fits-all opioid prescription crisis that is causing so much suffering, a profound shift is underway in how some Stanford Medicine scientists are studying and clinicians are treating chronic pain.

"The word 'pain' does not refer to one kind of thing we can — or should even try to — turn off with a single drug," said pain medicine specialist Vivianne Tawfik, MD, PhD, associate professor of anesthesiology, perioperative and pain medicine, who is one of 28 physicians practicing in the Pain Management Center at Stanford Health Care. "Pain is hundreds of different things. And we've learned that that's how we must treat it."

KOBILKA IS HOTLY PURSUING AN APPROACH THAT WOULD AMPLIFY AND FINE-TUNE THE BODY'S OWN PAINKILLING MECHANISMS.

The new approach, said Mackey, who leads the pain center and holds the Redlich Professorship, is to personalize pain treatments — "to fill our clinical buckets with the best tools we can make or find and then zero in on the best combination of them to use to help each individual patient for their unique pain problem."

Mackey was co-chair of the committee that produced the National Pain Strategy, a 2016 report funded by the National Institutes of Health, recommending a multi-modal approach to improving the assessment and care of people in pain. Mackey also was co-author of a recent Stanford-*Lancet* Commission report calling for sweeping reforms in response to the opioid crisis.

Pain research and treatment at Stanford Medicine ranges from the molecular to the psychological. One group of researchers seeks to hack the nervous system's pain networks with molecular compounds that more safely and subtly adjust the gain on pain. Another is experimenting with the mushroom-derived psychedelic drug psilocybin to forge new, more tolerable relationships to the pain they can't get to go away. And another educates patients through online instructional sessions about the physiological and psychological aspects of pain and how to manage it. Finally, a major NIH-supported effort aims to find biomarkers for pain and identify which patients will respond to which treatments.

WHAT IS CHRONIC PAIN?

The International Association for the Study of Pain defines pain as an unpleasant sensory and emotional experience associated with or resembling actual or potential tissue damage. Pain that persists or recurs for longer than three months is considered chronic.

If pain is an alarm announcing injury or its threat, chronic pain is an alarm that won't turn off, even if no injury or threat remains. In such cases, pain becomes its own pathology, not an indication of another. The depression, anxiety and sleep disorders that can result can make the pain worse, laying down a neural circuit of suffering that can get more and more difficult to interrupt. Chronic pain is also associated with many other disorders including cardiovascular and sexual ones. It is also tied to a higher-than-normal rate of suicide.

When you step on a tack, a kind of cell called a nociceptor detects the damage and sends a signal up your leg to your spinal cord, where it connects to another long nerve cell that takes it to the part of your brain called the thalamus, where it is perceived and then sent to the cortex, where it finally turns into suffering. "Ouch," you say, moving your foot off the tack.

Almost immediately, your brain sends signals back toward the injury: "Message received, turn off the alarm and calm everyone down." Pain-quelling chemicals are released along the entire path to do just that. If you've put a hole in your foot, the acute stab of pain will transition to a slower, lower-level ache that will diminish and fade as the wound heals.

If that injury is repeated, however, other pain signals may head brainward, where they are first perceived and then experienced as suffering. Long-lasting or repeated injuries or serious infections can cause chronic pain, and sometimes, even after an injury has healed, errant alarm signals continue to alert the brain about tissue damage that no longer exists.

THE SCIENCE OF MAKING PAIN STOP

Opioids are a powerful if blunt pain intervention. They turn off pain by interrupting the pain signals being sent to the brain. When you take morphine, the opioid molecules enter your bloodstream and spread around your body, fitting into little locked switches on the outsides of cells. These switches, proteins called receptors, unlock and turn on or off when they receive molecules of just the right shape and size to bind to their active site.

In the case of receptors that activate or inhibit nociceptors, they either send pain signals to the brain or keep those signals from being sent. The shape of the molecule that binds to the receptor defines the medicine. Molecules that fit into the main class of pain receptors, opiate receptors, are opioids.

The Holy Grail of pain treatment has long been a compound that fits into opiate receptors to turn off pain, without causing adverse side effects. The problem with opioids, of course, is that those same opiate receptors, in addition to turning off pain, can also send signals to stop breathing. Opioids also are addictive. That is a deadly combination responsible for more than 100,000 U.S. deaths each year.

More than a decade ago, Nobel Prize-winning work by Stanford Medicine physiologist Brian Kobilka, MD, made possible the search for a more targeted opioid painkiller, one that would quell pain without stopping breathing or causing addiction.

Kobilka, who's the Hélène Irwin Fagan Chair of Cardiology, and Nobel co-recipient Robert Lefkowitz, MD, were the first to describe in detail the large class of membrane receptor proteins known as G-protein-coupled receptors, which include opiate receptors. These receptor proteins receive messages on the outer surface of a cell that set off actions inside of it. They are the interface where cells receive most of their instructions, including the command to turn off pain.

The immense complexity of receptors and the molecules that unlock them has so far frustrated scientists' efforts to find a much safer yet still effective opioid. But, aided by new laboratory techniques, Kobilka is hotly pursuing an approach that would amplify and fine-tune the body's own painkilling mechanisms.

RETUNING THE BRAIN'S PAIN CIRCUITRY

Kobilka's strategy is to discover a drug that adjusts the receptor protein's sensitivity to the body's naturally produced opioids. This is not how classic painkillers work. Instead, they usually mimic the body's natural opioids, binding to the receptor's active site. Kobilka is seeking molecules that bind to another location on the same protein and remotely influence the active site's behavior.

This strategy, which is also being used by others, might sound indirect, but it makes sense when you think about how receptor proteins function. Proteins are masses of atoms that flex, twist and wiggle in response to their environment, and the binding of molecules anywhere on the protein's surface can trigger a change in the protein's shape and influence the sensitivity of an active site.

In drug discovery lingo, the active site is known as the orthosteric target, and a binding site that influences the orthosteric site is an allosteric target. Opioid drugs have traditionally targeted the obvious location: the receptor protein's orthosteric site. But Kobilka is shooting for an allosteric site.

"A megaphone is a pretty good analogy for the allosteric site," said Kobilka. "The source of sound — the voice in this analogy — comes from the orthosteric site. But if there is a voice, it can be modulated — turned up — by the allosteric site."

Kobilka wants to find an orthosteric binding site that can be activated only by the body's own endogenous opiates, so he can then target an associated allosteric site and amplify that already present pain suppression. Such a drug could be both more nuanced in the physiological changes it targets and also have a kind of built-in anti-abuse security system.

"If a person doesn't have a release of endogenous opiates as a response to their own pain," said Kobilka, "then the allosteric modulator won't do anything. If you aren't in pain, taking the drug would be like turning a megaphone up but with no sound to amplify."

New technology is allowing Kobilka and his colleagues to quickly search libraries of up to a trillion compounds for molecules that bind with specific allosteric sites in promising

ways. "These new libraries significantly broaden the chemical space we can search, making it much more likely that we will find something effective and safe," he said.

STUDYING CANNABINOIDS FOR MORE TYPES OF PAIN

In addition to hunting for precisely shaped painkilling molecules to engage opiate receptors in more targeted ways, Kobilka and his team are looking for molecules that engage another class of pain receptors altogether: cannabinoid receptors. Like opioid receptors, cannabinoid receptors are a class of G-protein-coupled receptors that, among other things, are involved in the experience of pain. Kobilka is collaborating with Tawfik and others on a cannabinoid-receptor-focused project funded by the Defense Advanced Research Projects Agency.

"Brian is 1,000% receptor-focused," said Tawfik. "His brilliance is looking at the structure of a receptor and being able to target compounds to that receptor in a way that engages it and causes the activation of different downstream pathways. … Then, once he's found interesting and promising ones, I see if they work in mice."

Another reflection of the shift toward making more targeted, personalized drugs is the fact that Tawfik has expanded the number of pain models she uses for her research compared to just a couple of years ago. Then, she and her team were focused on one model, for complex regional pain syndrome, a debilitating chronic nerve condition she specializes in treating. But they now have a half dozen different models for different kinds of pain, she said.

"For a long time, we were looking for the panacea; a compound that would just cure pain, period! So, we wanted it to work in every mouse model for every kind of pain. But that's exactly where we got into trouble," she said. "Now, if a drug doesn't work on one model, I see it not as a fail but as a promising sign that it might be targeted enough to be of real value for another pain condition."

'NOW, IF A DRUG DOESN'T WORK ON ONE MODEL, I SEE IT NOT AS A FAIL BUT AS A PROMISING SIGN THAT IT MIGHT BE TARGETED ENOUGH TO BE OF REAL VALUE FOR ANOTHER PAIN CONDITION.'

Tawfik would rather be able to prescribe a drug for a patient with inflammatory pain, say, that addresses only inflammatory pain and nothing else. "You don't want to turn all pain off, you just want to adjust that one disabling pain," she said.

By definition, chronic pain is persistent, which is why Mackey says it is best addressed on multiple fronts at once. "If Brian Kobilka finds a nonaddictive non-respiratory-depressing opioid, that would be an incredibly important tool," said Mackey. "But would it cure pain? Absolutely not!"

DEVELOPING A MULTIPRONGED APPROACH

"Today, pharmacology is just a small part of what we do," said Mackey. "Our pain center also employs psychological approaches, physical and occupational therapy, complementary and alternative medicine, and patient empowerment or educational approaches."

Researchers are taking many tacks as well.

A Stanford Medicine researcher honing an educational approach is pain scientist and psychologist Beth Darnall, PhD: After years of teaching multisession pain management classes that have been studied for decades, she has compressed key elements of the classes and combined them with other material into a widely accessible two-hour class. The intervention, called Empowered Relief, gives people with acute and chronic pain neuroscience education, mindfulness-based principles and some cognitive behavioral therapy-based skills to better manage their pain and related symptoms, said Darnall, a professor of anesthesiology, perioperative and pain medicine and director of the Stanford Pain Relief Innovations Lab.

The program launched in 2013 and in 2019 was disseminated to people with acute and chronic pain in 16 countries and in seven languages.

"Most of the 100-million-plus people in the U.S. living with pain don't have easy access to surgery or carefully managed pain medications or an eight-session cognitive behavioral therapy to gain pain management skills," she said, noting that Empowered Relief expands access to pain care, and it can work alongside other treatments.

The course is offered free by some health care providers (including Stanford Health Care), and certified instructors are listed online at stan.md/paincourse, said Darnall.

"Data from multiple trials show that we can help significantly reduce pain and other key outcomes with this one-time intervention," she said.

A study published in *JAMA Network* last year favorably compared the effectiveness of Empowered Relief to eight-session cognitive behavioral therapy for people with chronic

lower back pain. Participants in both groups showed significant reductions in "pain intensity, pain interference, sleep disturbance, anxiety, depression and pain bothersomeness," said Darnall. Another study published this year in *Anesthesia & Analgesia* showed that orthopedic trauma surgery patients who received a version of Empowered Relief had reduced pain after surgery and up to three months later. Cleveland Clinic offers Empowered Relief in its chronic pain clinic and as standard medical care for all spine surgery patients.

Research that bridges molecular and psychological approaches is also unfolding in the lab of Boris Heifets, MD, PhD, who is studying the use of psilocybin for chronic lower back pain. While some evidence indicates that psychedelics like psilocybin may have a direct analgesic effect, Heifets said, the point of his research is to see if a psilocybin trip helps some people improve their relationship to their chronic pain.

An experimenter stays with each subject through the experience, remaining nearby for the subject's safety and peace of mind but not engaging in psychotherapy during the trip. Heifets wants to establish whether a baseline effect exists from only ingesting the drugs before adding psychotherapy to the sessions.

"The psychological component is an essential part of recovery from chronic pain," said Heifets. "Psychedelics can catalyze psychological transformations that could take a very long time without a chemical catalyst."

MATCHING PATIENTS AND TREATMENTS

Whether Stanford Pain Management Center specialists employ medications or other treatment options, relieving a patient's chronic pain depends on their ability to personalize that care.

"The difficult part is figuring out which ones will work for which patients," said Mackey. He has been treating pain patients for decades but, he said, "even with all my experience, I can only predict which treatments will work for any given patient 30 to 40% of the time."

One of the most effective drugs for some chronic pain patients, for example, is naltrexone, a drug initially designed to block the intoxicating effects of alcohol and drugs and to reduce cravings. Researchers believe it has an entirely different action at lower doses, reducing neural inflammation and pain.

"It is a hit-the-ball-out-of-the-park drug that is transformative for many people with chronic pain. It has almost no side effects. And it is dirt cheap," said Mackey, who collaborated on some of the original experiments on low-dose naltrexone for chronic pain. "The problem is, I don't know which of my patients it's going to work for until I try them on it for a few weeks."

But Mackey said he and his colleagues are chasing a solution to the challenge of matching chronic pain patients and therapies by developing objective biomarkers to enable a precision medicine approach for pain. His team is deploying brain imaging technology, genetics, wearables, sensory testing, self-reporting surveys and machine learning models to predict the treatments — including medications — a person suffering from chronic pain will best respond to.

They have also created a learning health system called CHOIR that compiles high-quality data from every patient encounter. Clinicians have implemented the system at clinics at Stanford and throughout the United States.

"CHOIR allows us to characterize the unique profile of every patient in ways that help us target the right treatments and to track them over time," he said. "Take naltrexone, for example: If instead of that 30-to-40% rate, I could select with 90-to-100% accuracy, that would make all the difference."

Ultimately, Mackey plans to integrate the biomarker development work into CHOIR to aid clinical decision-making and improve the outcomes of patients with pain and other conditions.

One young female patient with a debilitating case of complex regional pain syndrome sought Mackey out after years of failing to improve under her local care in Florida.

"Using CHOIR, we could see that in addition to her pain, she'd also had terrible fatigue, poor sleep and depressed mood — an association of symptoms that all can respond to low-dose naltrexone," said Mackey. "Once on the drug, she started sleeping better, feeling less tired and less depressed. Her pain showed dramatic improvement too."

When she felt well and attentive enough to get some traction in pain psychology, Mackey enrolled her in Darnall's Empowered Relief class, which further amplified the improvements. "While still not entirely pain free," said Mackey, "she got much more energetic, focused, hopeful and able to pursue a better future.

"Now she works, has two young children and is enjoying life. It would have been hard to imagine any of that when she first came to us."

From data to molecules to psychology, welcome to the new and improved world of chronic pain treatment. **SM**

— Contact Gordy Slack at medmag@stanford.edu

Read about a Stanford Medicine-led report on the opioid crisis that was released in coordination with *The Lancet* at stan.md/opioids.

FOLLOWING HER DAUGHTER'S DEATH, A MOTHER'S DONATION HELPS OPEN THE DOOR FOR CANCER IMMUNOTHERAPY

A GIFT OF HOPE

BY ERIN DIGITALE

PHOTOGRAPH BY TIMOTHY ARCHIBALD

WHEN MYCAH CLEMONS' 4-year-old daughter died of a brain tumor in 2014, Clemons wanted to help others affected by the disease that took Mayianna's life — a fast-growing cancer called diffuse intrinsic pontine glioma, or DIPG. Clemons donated the tumor to the Stanford Medicine lab of Michelle Monje, MD, PhD, professor of neurology and neurological sciences, whom she had contacted months earlier for advice on her daughter's case. During that conversation, Monje had asked Clemons to consider providing the tumor for research. Monje's team was the first in the world to culture donated tumors and study the cells directly.

"Talking to Dr. Monje, that's when I learned that tumor donation was essential to finding out more about DIPG," Clemons said. DIPG tumors affect a few hundred children in the United States each year and have a grim prognosis, with a five-year survival rate below 1%. Yet Clemons had mixed feelings about donating the tumor: The idea that the aggressive tumor could continue to exist after Mayianna's death disturbed her. "Especially with a child so young, it's hard to process," she said.

Facing a tough decision

CLEMONS' CHOICE TO DONATE WAS DRIVEN BY a steadfast desire to enable scientific advances that might prevent families from losing a child the way she did. She also led efforts to raise about $6,000 for Monje's lab by hosting events such as a fashion show and a dance for friends and family in Pittsburgh and by selling DIPG awareness merchandise on a website she founded. Clemons, her family and her community are still raising funds to support the lab, hoping to reach a total of $30,000.

Since 2009, Monje's team has received 87 DIPG tumor donations, allowing them to study the malignant cells in the lab and in animal models and to share DIPG cells with scientists around the world. For each donated tumor, the scientists coax live cells from dead debris, culture the living cells in baths of liquid cell food, then put them to work for experiments. The research has revealed unique DIPG cell features that may be good cancer-treatment targets, which could be revolutionary for a disease that currently has no effective chemotherapy.

Clemons' financial donation funded a summer scholarship that allowed an undergraduate student in Monje's lab, Evan Arnold, to undertake a key project in 2016: He screened DIPG cells to see what molecules protruded from the cell membranes, showing that the cells abundantly display a molecular marker called GD2.

"It was a big surprise because it's not a protein," said Monje, noting that most research on brain cancer cells' identifying markers has focused exclusively on proteins. GD2 is a ganglioside, made of two long fingers of fat that embed themselves in the cell membrane, anchoring a complex, lumpy sugar that sticks out from the cell. If Arnold had used the other screening methods — such as looking at data from frozen tissue samples that indicates protein production — instead of studying living cells from donated tumors, the discovery would have been missed.

Gangliosides' roles are just starting to be understood. The body normally uses GD2 judiciously, putting small amounts on certain nerves and brain cells as a "don't eat me" signal to the immune system. But scientists have found much more GD2 on some cancer cells, which suggests it could be targeted for cancer treatment. In fact, at the time of Arnold's project, cancer immunotherapy expert Crystal Mackall, MD, professor of pediatrics and of medicine, had already engineered an anti-cancer immune cell known as a chimeric antigen receptor T cell, or CAR-T cell, to target GD2.

"We knocked on her door and said, 'You have a CAR-T cell that targets this?'" Monje said. They teamed up and showed the cells could make DIPG tumors disappear in mice.

Now the team is testing the effects of anti-GD2 CAR-T cells on people with brain and spinal cord tumors. Though the trial's first four patients eventually died of their disease, their experiences showed that it's possible to reverse severe debilities caused by the tumors. CAR-T cells helped the trial's second patient, a young man named Jace Ward, temporarily regain an almost-normal gait and the ability to open his mouth after the tumor left him struggling to walk and eat. "He went in in a wheelchair and walked out of the hospital," said Jace's mom, Lisa Ward, recalling her son's treatment with CAR-T cells. "It was so freeing for him, such a good glimmer of hope."

The discoveries that began with donations from Clemons and other bereaved families give Monje a lot of hope.

"Mycah worked for a year to come up with the funds for Evan's project, and this is what it turned into," Monje said. Clinical trials continue, with the scientists refining how the powerful immune cells can help patients.

"I'm really proud that they're creating something all DIPG families want: the opportunity to have something promising when they reach out to a doctor," said Clemons. "It's bittersweet … but to see it happening is so exciting."

Recalling how she felt when anti-GD2 cells first reversed Jace's symptoms, Monje said, "I felt for the first time that we were going to be able to cure this disease someday." **SM**

— Contact Erin Digitale at digitale@stanford.edu

MICHELLE MONJE IS SEEKING WAYS TO FIGHT BRAIN CANCER BY STUDYING CELLS GROWN FROM PATIENTS' TUMORS.

WHEN PHLEGM RUNS AMOK, IT CAN BE LIFE-THREATENING. NEUROSCIENCE KNOW-HOW OFFERS A WAY TO PUT A CORK IN IT.

THE MIND-MUCUS CONNECTION

BY BRUCE GOLDMAN

ILLUSTRATION BY MELINDA BECK

Next time it feels like you're sneezing your brains out or coughing up a lung, consider that brains and lungs have something very much in common: They share some secrets about secretion. Nerve cells in the brain take the high road, emitting bursts of chemicals in order to pass their signals from one to the next (a process known as neurotransmission). Goblet cells in the lung take the low road, squirting out rivers of mucus when they get irritated.

Yep, mucus. Like it or lump it, we can't live without it. We don't think too much about the sometimes slimy, sometimes sticky, sometimes lumpy stuff, and when we do, we don't think much of it. But our health hinges on it.

In the right amounts, at the right consistency, mucus is a lung's best friend. It's also essential to the proper function of the stomach, intestine and urogenital tract. But if there's too much of it, or if it's too adhesive, it can betray the organ it is meant to serve.

Take the lungs, for example. Airway blockage by overly sticky mucus is a hallmark of several serious respiratory disorders and a major medical problem. In the United States alone, more than 25 million people have asthma and about 2.5 million of them are unable to find relief from existing drug treatments. Medications for chronic obstructive pulmonary disease, which also affects about 25 million in the United States, are even less likely to work. Excess mucus also spells trouble for the 1 in 20 people who develop acute bronchitis each year. And for the

roughly 30,000 Americans who have cystic fibrosis.

Oddly, the excessive buildup of mucus in the lungs and airways bears some powerful resemblance, at the molecular level, to the way nerve cells in the brain secrete pulses of specialized chemicals to transmit signals to one another. In a sense, these chemicals, called neurotransmitters, form the substrate of our soul: They guide our every cognition, emotion, motion and ambition.

To carry out their lofty job description, neurotransmitters need to be released in a precise manner. Mucus, not so much — "precision mucus" is not a thing. But there's still a big difference, healthwise, between just enough of it and way too much of it.

Any resemblances between neurotransmission and mucus hypersecretion do not extend to the attention they get from researchers. Walk the halls of any solid medical school, and you will come across departments of neurology, neurobiology, neurosurgery and psychiatry. If you were to stumble on a department of mucus, your first instinct would probably be to pick up your pace or to attempt to wake up.

It stands to reason that the obvious importance of a functioning nervous system would result in quite a lot of research attention being focused on neurotransmission. Mucus secretion, although vitally important, is less glamorous, far more esoteric, and more quietly explored.

But the burden of medical disorders caused or exacerbated by too much mucus is nothing to sneeze at. As fate would have it, hard-won insights from the world of neuroscience are now directing beams of understanding at lung disorders caused by too much mucus.

Using techniques originally designed to tease apart the functions of several proteins that work together to coordinate the release of neurotransmitters, a team including a neurotransmission expert and a mucus explorer has measured and modified the workings of the pathway responsible for excessive release of a key protein in mucus. This may soon pay off in the form of entirely new, precisely targeted treatments for mucus-stressed lungs.

The leader of the project, Axel Brunger, PhD, a Stanford Medicine professor of molecular and cellular physiology, of neurology and neurological sciences and of photon science, has a history of research delineating the workings of neurotransmission. In the course of his career, he has collaborated frequently with Tom Südhof, MD, a professor of molecular and cellular physiology who received a Nobel Prize for demonstrating how myriad tiny bubble-like packets, or vesicles, containing neurotransmitters are held in just the right place inside a nerve cell, ready to release the signal-bearing molecules at just the right moment.

Armed with this understanding, Brunger, other Stanford Medicine investigators and collaborators at the University of Texas MD Anderson Cancer Center in Houston and at Ulm University in Germany have designed a compound that's capable of blocking mucus hypersecretion while, crucially, not interfering with the necessary low-level secretion of the gummy substance.

"This is the first compound that specifically alleviates the pathological hypersecretion of mucus common to cystic fibrosis, chronic obstructive pulmonary disease, asthma, viral respiratory infections and more," said Brunger, who is a Howard Hughes Medical Institute investigator.

The discovery, described in a study published in March in *Nature*, could improve the lives of millions who suffer from airway obstruction caused by excess mucus.

What we call breakthroughs seldom come about by a burst of insight. They more often unfold inch by painstaking experimental inch, sometimes accelerated by the serendipitous intersection of two minds.

In landmark papers published in the past 25 years, Brunger and various co-authors (including Südhof) discovered the myriad molecular details of how a select group of proteins cooperate to orchestrate neurotransmission.

Burton Dickey, MD, a mucus expert and professor of pulmonary medicine at the MD Anderson Cancer Center, has in years past collaborated with Südhof on neurotransmission research. Work by Dickey and other scientists has shown that upsized secretion of mucin — a long, stringy protein that's

mucus's distinguishing component — works in much the same way as neurotransmission, involving analogous vesicles and collaborating proteins. One crucial difference: The vesicles are filled with mucin instead of neurotransmitters.

To advance his neurotransmission research, Brunger invented a technique that enables him to observe how adding selected proteins to stripped-down laboratory models of single vesicles affect a vesicle's behavior — for example, in relation to a similarly stripped-down and tweaked version of a cell's outer membrane. This method would come in handy in unexpected ways.

In May 2016, Brunger gave a lecture at the Baylor College of Medicine in Houston. In the audience sat Dickey, who'd heard about Brunger's work from Südhof, a professor at the University of Texas before coming to Stanford. Dickey approached Brunger after the lecture, told him he wanted to develop a compound to selectively block mucin hypersecretion — that is, to stop it in its tracks without interfering with the constant low-key output of mucin that ensures adequate mucus levels for proper organ function — and asked him if he'd care to collaborate. They became co-senior authors of the study.

MUCUS FACTS

Mucus may not get talked about much at swank soirees, but there it is, lurking inside of every guest. It comes in various colors and viscosities, and hails from various regions of the body besides the lungs. There's plenty of it in the nose. Saliva is mostly mucus. You also find it in the stomach and gut. (Mucus expelled from the lung is known as phlegm, or sputum.)

Different microbes impart different colors to mucus. Some molds are black, and they can turn mucus black. Blood makes it dark red.

But superficial differences aside, there are two main ingredients.

A healthy respiratory system's mucus is 97% water. The other main ingredient, mucin, is secreted by goblet cells and seromucous glands at or just below the surface, or epithelium, of the nose, throat, bronchial tubes and small airways in the lungs. Lengthy chains of sugar molecules sprout from each mucin molecule's surface, predisposing the protein to absorb water.

Mucin molecules readily cross-link into networks, forming the viscous gel we know so well. Secreted at moderate levels, mucus coats airway surfaces, serving as a lubricant and a protective barrier as well as a straitjacket for encapsulating microbial pathogens. The encased microbes are driven out of the lungs and upward in the airways by hair-like structures called cilia that project from cells abundant in the airway lining.

Few of us spend any time pondering these minutiae because a steady hum of crucial mucus secretion goes on pretty much all the time. We hardly notice it — until a fly lands in the ointment. Or until a microbe lands in the lungs.

Inflammation, often elicited by microbial pathogens, shifts mucin secretion into overdrive. That's great for trapping and washing away offending microbes, but persistent inflammation can cause trouble. What's more, the newly secreted inflammation-triggered bolus of mucus often contains a higher-than-normal mucin content, increasing the liquid's viscosity. The thickened mucus can congeal into rubbery pads, or plaques, stifling gas exchange in the lungs and impeding airway cilia's upward pumping of mucus-entrapped pathogens.

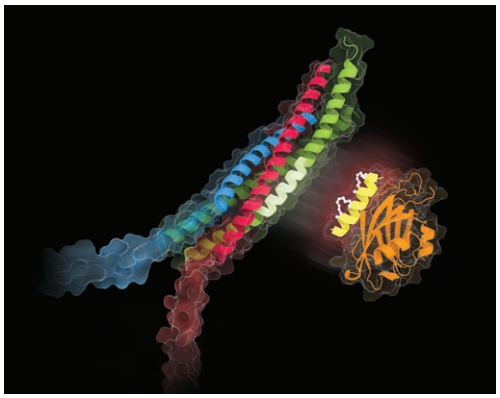
SECRETION SECRETS

Evolution, having devised a nifty trick, would be remiss to relegate its execution to a single instance or an individual organ. Secretion of neurotransmitter chemicals in the brain and hypersecretion of mucin in the lungs work similarly. In fact, some scientists think neurotransmission and mucus secretion may have evolved from the same ancient pathway — it's also found in glue-secreting cells studding the tentacles the comb jelly, a jellyfish cousin, uses to capture plankton.

NEUROTRANSMISSION — THE RELAYING OF IMPULSES FROM ONE NERVE CELL, OR NEURON, TO THE NEXT — DEPENDS ON THE CAREFULLY TIMED SECRETION OF NEUROTRANSMITTERS FROM NEURONS' TIPS.

As we've seen, big bursts of mucin secretion and subsequent mucus buildup are driven by abnormal events such as inflammation. In the brain, analogous bursts of high-level secretion, far from being outliers, are critical to nerve cells' normal function: signaling.

Neurotransmission — the relaying of impulses from one nerve cell, or neuron, to the next — depends on the carefully timed secretion of neurotransmitters from neurons' tips. Those chemicals are routinely caged inside tiny vesicles situated near a neuron's surface membrane like containers on a ship, waiting to be unloaded.



AN ENGINEERED PEPTIDE (BRIGHT YELLOW) INHIBITS MUCIN SECRETION BY DISRUPTING THE INTERACTION BETWEEN SYNAPTOTAGMIN (ORANGE) AND A COMPLEX OF COOPERATING PROTEINS (BLUE, RED AND GREEN).

Spontaneous merging of these vesicles' walls with the neuron's outer membrane happens all the time, resulting in a fairly constant, low-level expulsion of the vesicles' stored contents from the neuron. But electrical impulses traveling through the neuron trigger an abrupt, temporary influx of calcium into the neuron. When calcium binds to a protein called synaptotagmin on the vesicles' surfaces, synaptotagmin teams up with other proteins to tug some of the vesicles, winch-style, closer and closer to the neuron's surface membrane until their enclosing membranes fuse with it, spilling the vesicles' contents into the surrounding environment. The chemicals can then diffuse to nearby neurons, exciting or inhibiting activity in those recipient cells.

A CLOSER LOOK AT SYNAPTOTAGMIN

In the new study, Brunger's team showed that synaptotagmin is also essential to the rapid large-scale fusion of mucin-containing vesicles with secretory cells' outer membrane — and to the resulting massive mucin expulsion. And in studies in mice, they found that eliminating synaptotagmin dialed down mucin release considerably in inflammatory conditions that typically generate buckets of it, along with allergic reactions. If reducing the presence of synaptotagmin had this effect, then blocking its activity was clearly an approach worth exploring.

Brunger's and Dickey's groups designed a small protein snippet, or peptide, called SP9, that blocked synaptotagmin's calcium-triggered interaction with its cooperating proteins in mice. SP9 is essentially a synthesized segment of a protein to which synaptotagmin binds. To stabilize SP9's shape and ensure its effectiveness, the team bolstered the peptide with biochemical braces.

The study's lead author, Ying Lai, PhD, then a postdoctoral scholar in Brunger's lab, applied Brunger's single-vesicle-modeling technique to mucin-containing vesicles and mucin-secreting cells' outer membranes — the classic Brunger approach. In this simplified system, SP9 preferentially inhibited inflammation-driven hypersecretion of mucin, mucus's chief protein constituent.

But the researchers needed to find a way for SP9 to transit the secretory cell's outer membrane and get inside, where the action is. Working with Manfred Frick, PhD, a professor of medicine at Ulm University who became the study's third co-senior author, Lai solved the problem by splicing SP9 to yet another peptide, PEN, that's known to be excellent at penetrating cell membranes. Frick's group confirmed, in human cells cultured from lung-tissue biopsies, that the conjoined-peptide pair penetrated the secretory cells and blocked their inflammation-induced mucin secretion. Dickey's lab at MD Anderson then showed that PEN-SP9 administration in mice not only stymies inflammation-induced mucus secretion but also substantially reduces the plaque-plugged area in the mice's lungs. That suggests it could be effective as a therapy in humans.

TOWARD CLINICAL TRIALS

Brunger and Dickey have filed a joint provisional patent application with Stanford's Office of Technology Licensing and the MD Anderson Cancer Center's Office of Technology Commercialization. Brunger hopes to see an optimized version of SP9 begin testing in patients within the next two to three years.

"This didn't happen by accident," Brunger said. "It's been a long road with many failures, and it shows the importance of basic research — you just keep putting one foot in front of the other, and then suddenly you have something." SM

— Contact Bruce Goldman at goldmanb@stanford.edu

ERIC D. SMITH

A GUIDE THROUGH THE CANCER LABYRINTH

TOWARD A PEER NAVIGATION PROGRAM FOR BLACK WOMEN HELPING BLACK WOMEN SURVIVE BREAST CANCER

By Ruthann Richter

PORTRAITS BY TIMOTHY ARCHIBALD

JUANITA WAUGH had the best of medical care when she had breast cancer in 2005, but she had little guidance for life outside of doctors' offices. As a result, she struggled through a nightmare of side effects, wildly shifting emotions and fear of what could happen next.

No one told her what to expect from the treatments, so skin burning and discoloration from radiation and the foggy brain after chemotherapy came without warning. Nor did anyone advise her on more practical things, like how to find a wig that would make her feel more like herself after chemotherapy caused her to lose most of her hair.

"I knew nothing about breast cancer — nothing whatsoever," said Waugh, a retired health insurance worker who lives in Oakland, California. "When I was going through the process, I felt like I was in a dark tunnel looking for the light."

Now, Waugh is one of a group of Black women on a steering committee for a project designed to ease treatment and recovery for Black women with breast cancer and increase their odds of survival, which are significantly lower than those for white women. The project is sponsored by Stanford Medicine and the California Breast Cancer Research Program.

Black women in the United States are diagnosed with breast cancer at the same rate as white women, yet they are

STARLA GAY, JUANITA WAUGH, LENORA WILLIAMS-OMENKA AND CHIQUITA TUTTLE (FROM LEFT TO RIGHT) ARE AMONG A CADRE OF WOMEN WHO INTERVIEWED BLACK BREAST CANCER SURVIVORS ABOUT THEIR EXPERIENCES WITH THE DISEASE.

40% more likely to die from the disease and twice as likely to die from it if they are older than 50, according to American Cancer Society data. In the United States, some 36,260 Black women are expected to be diagnosed with the disease this year, resulting in about 6,800 deaths, the cancer society estimates.

The aim of the project is to build a model peer navigation program in which Black women who have experience with the disease — personally or through a family member — guide others through the labyrinth of cancer care. They can help sort through treatment options, address practical concerns such as child care and transportation, point to available resources and offer compassion, emotional support and a listening ear. The project's researchers are shaping the program using information they've gathered from Black breast cancer survivors.

Lisa Goldman Rosas, PhD, a Stanford assistant professor of epidemiology and population health and of medicine, is co-leading the project with Starla Gay, a longtime community organizer and advocate. They plan to establish a program in Alameda County, the Bay Area county with the largest Black population, at 11%, while learning more about how to improve the peer navigation process.

LISA GOLDMAN ROSAS, LEFT, AND STARLA GAY CO-LEAD THE PEER NAVIGATION PROJECT THAT IS GEARED TOWARD REACHING BLACK WOMEN WHO ARE NEWLY DIAGNOSED WITH CANCER TO HELP THE WOMEN HAVE BETTER HEALTH OVERALL AND BETTER TREATMENT OUTCOMES.

"The idea of having a peer navigator is that you have someone who is positioned to take you through all the different pathways of the disease, be it a medical pathway, educational pathway or alternative pathway, such as holistic interventions," Gay said.

"If we can catch women when they've been diagnosed and enroll them in a peer navigation program, that will improve their health overall and may decrease the likelihood of poor outcomes."

The reasons Black women are more likely than white women to die from breast cancer are complex and not well understood, said Rosas, who is the faculty director of Stanford Medicine's Office of Community Engagement.

One theory is that genetics may be at play, with some studies suggesting that Black women may be genetically predisposed to some more aggressive breast cancers. They also are more likely to develop triple-negative breast cancer, which is particularly hard to treat, according to the Breast Cancer Research Foundation.

Adana Llanos, PhD, an epidemiologist and geneticist at Columbia University who has studied health disparities in cancer, said studies suggest social factors are also important contributors to the dire outcomes for Black women.

Like racial and ethnic minorities, generally, Black women are more likely than white women to live in low-income areas that are "food deserts" with poor dietary options and lack green space where they can exercise without fear of crime, she said. They also are less likely to have health insurance or access to medical facilities with advanced technologies for care, she said.

"I don't think there is necessarily a genetic explanation for why Black women are more likely to die of breast cancer," said Llanos, who serves on the project's community advisory board. "It's the other external things that impact

'I DON'T THINK THERE IS NECESSARILY A GENETIC EXPLANATION FOR WHY BLACK WOMEN ARE MORE LIKELY TO DIE OF BREAST CANCER. IT'S THE OTHER EXTERNAL THINGS THAT IMPACT US — THE SOCIAL DETERMINANTS OF HEALTH.'

us — the social determinants of health."

She said research has also shown that Black women are more likely to suffer from chronic, lifetime stress that can negatively influence how they respond to breast cancer and how well they fare in the long run.

One study by the National Health and Nutrition Examination Survey showed that Black women had measurably higher levels of stress hormones and other biomarkers that could affect their quality of life and cancer outcomes. The study, published in 2012 in the journal *Psycho-Oncology*, found no comparable markers in white women.

"That stress relationship is really strong among Black women and almost nonexistent for white women," Llanos said.

In a study published in 2020 in the journal *Breast Cancer Research and Treatment*, Llanos found that two years after a breast cancer diagnosis, Black women had higher levels of stress that translated into poorer quality of life measures, such as pain, lack of energy, mental distress, poor sleep and reduced ability to function, compared to their white counterparts.

She said peer navigation has been shown to make a difference for these women. For instance, a paper published in March 2022 said it's been proven that patient navigation can lower costs, reduce hospital readmissions and emergency room visits, and improve quality of life for women with breast cancer.

The paper, published in the journal *Obstetrics and Gynecology of North America* by Cleveland Clinic researchers, noted that the concept of patient navigation was introduced in 1990 specifically to help Black women in Harlem Hospital who had late-stage breast cancer. Peer navigation is a form of patient navigation, with peers who have experience with a disease serving as the navigators.

DEVELOPING A PEER NAVIGATION PROGRAM

THE NEW PROJECT grew out of 2018 research that aimed to engage Black women interested in heightening awareness of cancer in their community. Through the project, 16 Black women in Alameda County organized community awareness events, such as presentations at churches and sororities. They reached more than 7,000 women during the yearlong project, which was supported by a $50,000 Innovation Award from the Stanford Cancer Institute and the Dr. Ellie Guardino Research Fund. Then, in 2019, they presented their work at the annual meeting of the American Association of Cancer Research, held in San Francisco.

Some of the women became so passionate about the cause that they chose to continue their advocacy, calling themselves Black Ladies Advocating for Cancer Care, or BLACC. Working with Rosas, the group obtained a $150,000 grant from the California Breast Cancer Research Program for the current project and formed a steering committee to design it, based on community needs.

"I strongly feel that community-based organizations hold the solutions and answers to their problems, rather than Stanford telling them, 'This is what you should do,'" Rosas said.

There are other breast cancer peer navigation programs in the United States that are geared toward Black women, but most tend to be based in health care institutions rather than in community settings that women may find easier to access and more user-friendly. Moreover, the program being developed by the BLACC project is unique in that it's being shaped by community members, Rosas said.

To gain insight into designing a program that addresses what newly diagnosed women need most, the team recruited a second group of 16 Black women — breast cancer survivors in Alameda County willing to discuss their cancer experiences. But rather than simply interviewing them, either individually or in groups, the project members tapped into the long-standing oral tradition in Black culture of storytelling, Rosas said, asking each woman to share her own history and lived experience. The group thought this approach might help the women feel more comfortable and encourage them to open up about their cancer journeys, Rosas said.

The group developed interview prompts, formulated some general questions, conducted story-gathering sessions on Zoom and then began analyzing the results. Once the initial analysis is completed this summer, they will develop a report that they hope will form the basis for a pilot program.

Steering committee member Chiquita Tuttle, PhD, who conducted some of the Zoom sessions, said she prepared questions designed to discover what the women knew about cancer before their diagnosis, what kind of treatment options or counseling they were offered, and whether they felt there was racial bias in their encounters.

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HERE COME THE ASSEM

A NEW TYPE OF BRAIN MODEL REVEALS THE ORGAN'S WORKINGS IN UNPARALLELED DETAIL, PROVIDING INSIGHTS INTO DEVELOPMENT AND DISEASE

By Bruce Goldman

PHOTOGRAPHY BY TIMOTHY ARCHIBALD

SERGIU PASCA WAS NERVOUS.

Let us count the reasons why.

Because of a pandemic-enforced hiatus, he hadn't spoken publicly — and indeed, like many of us, had greatly curtailed public contact — for two years. Now, on the morning of April 12, Pasca, an associate professor of psychiatry and behavioral sciences at Stanford Medicine, was giving a TED talk in Vancouver before an audience of about 1,500. Hectobillionaire Bill Gates had preceded him by a couple speakers. And he'd been instructed to establish deep eye contact with the strangers in the front row. (That really freaked him out, he said.)

He may have been making the audience a tad nervous too. He was telling them about a discovery guaranteed to raise eyebrows and blood pressure: how to turn anyone's skin into a replica of a small portion, or portions, of that person's brain, thriving and growing inside a lab dish.

Pasca, the Bonnie Uytengsu and Family Director of the Stanford Brain Organogenesis Program, assigns a fair share of credit for this feat to the brain. "The human brain largely builds itself," he said. "It comes with its own assembly instructions."

Which is fortunate. If, as rumored, the brain really is the most complicated thing in the universe, we're awful lucky we don't have to build our own. Of course, if you're trying to get one to grow in a dish, you are going to have to do some prompting. You can't just pour in some skin cells, add water and expect the little blob to respond with a hearty, "Thanks, I'll take it from here." You'll need to fold in a few key ingredients to coax the component parts of that most heterogenous of all organs, the brain, into existence and cooperation.

SERGIU PASCA IS PIONEERING THE CULTURING OF CELLS TO MAKE MODELS OF PARTS OF THE BRAIN.


BLOIDS

PASCA GETS CREDIT for figuring out the ingredients and conditions that encourage distinct parts of the brain to self-assemble as clusters called organoids in laboratory glassware. Now he has taken it a step further, coaxing the organoids to merge into agglomerations he calls "assembloids," in which cells migrate or extend projections from one organoid to the next, establish connections and build circuits that are credible, accessible working models of their mostly inaccessible real-world counterparts.

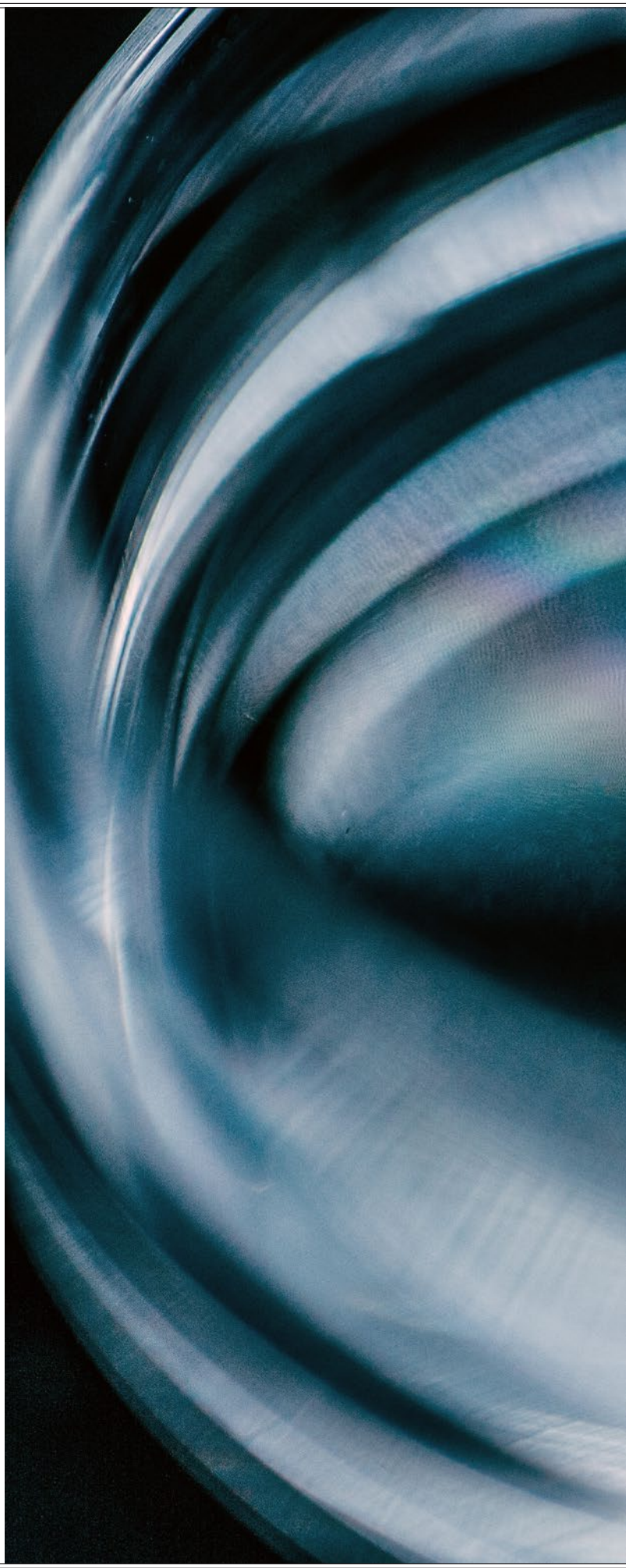
That lets researchers get action close-ups of the nearest thing to a living human brain that's ever come along without needing brain tissue from anyone living or deceased. They can learn how a healthy brain develops in a fetus or maybe even a newborn. And they can detail the deficiencies that derail brain function and cause neurodevelopmental disorders such as autism, schizophrenia and epilepsy.

Pasca arrived at Stanford Medicine in 2009, an MD from Romania with little training as a researcher or, for that matter, fluency in English. Now here he was, rubbing elbows with the likes of Gates, Elon Musk and more, and broadly considered the pioneer of an approach that's since been adopted by well over a hundred labs around the world, including those of several Stanford collaborators.

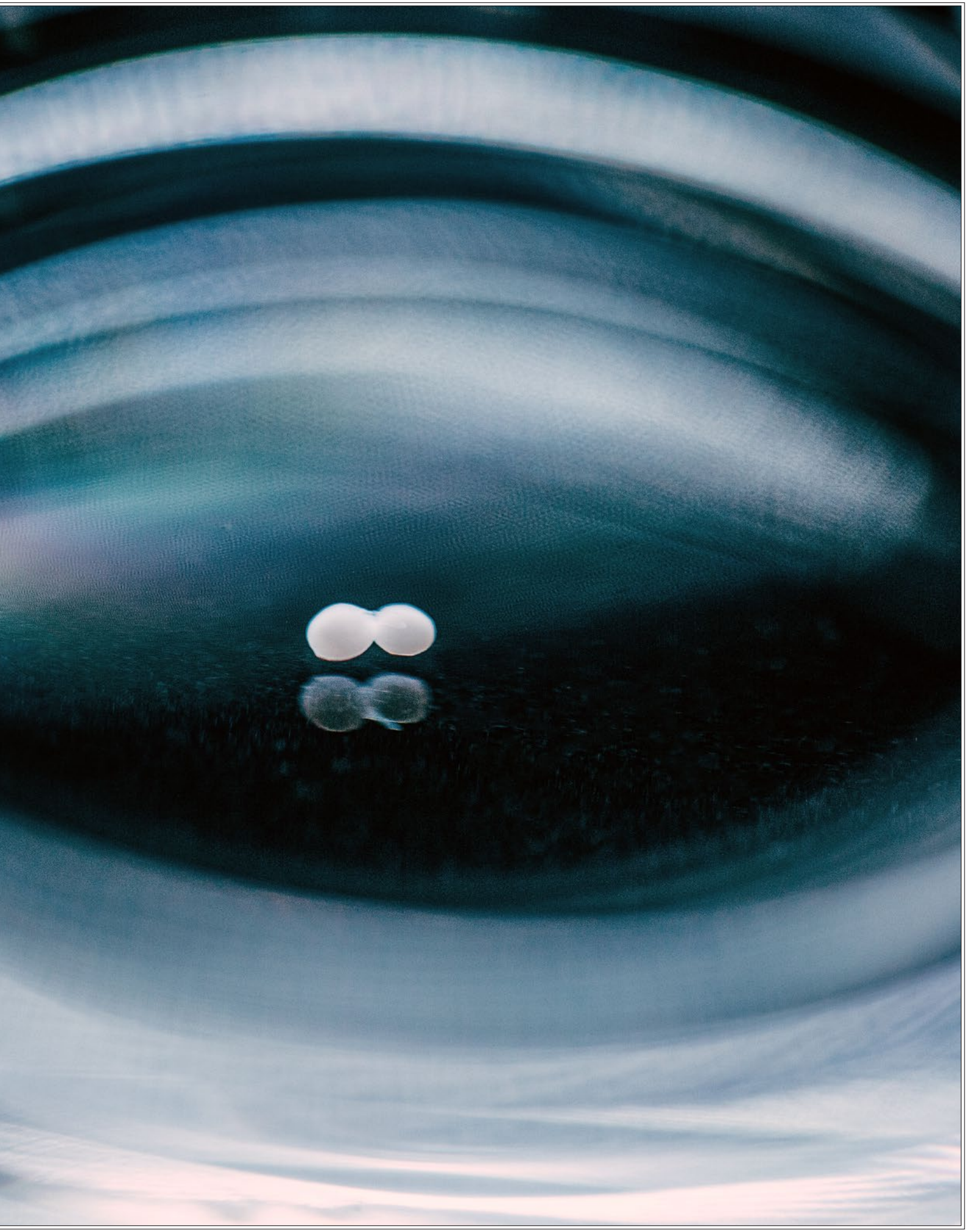
Stanford has licensed the methodology to a private company, Stem Cell Technology Inc. If you're a neuroscientist and you want to study the workings of one of the brain regions — or a combination of them — Pasca's lab has figured out how to make, you can buy a kit to do it yourself. You get starter parts plus reagents plus instructions.

A new era of brain research — what Pasca likes to call "molecular psychiatry" — has dawned. It promises new revelations about our most mysterious organ's inner workings and once-opaque development. It offers the prospect of living laboratories in which to test pharmacological and electrophysiological methods of curing nervous-system disorders that arise during early development or from infections and injury later on.

And it will catalyze not only comprehension and cures but also conundrums, as the products of Pasca's innovation inch forward to ever-increasing complexity, and start to resemble parts of our own brains in ways that are bound to concern us.



ROUGHLY THE SIZE OF A LENTIL, THIS ASSEMBLOID WAS FORMED BY THE JOINING OF TWO ORGANOIDS.



IT OFFERS THE PROSPECT OF LIVING LABORATORIES IN WHICH TO TEST PHARMACOLOGICAL AND OF CURING NERVOUS-SYSTEM OR FROM INFECTIONS AND INJURY LATER ON.

His TED talk — 13 years of research condensed into a 14-minute presentation — was interrupted more than once by applause.

THE RISE OF THE ASSEMBLOIDS

HERE'S A WHIRLWIND RECAP of what took place during that 13-year period and the three-year gestation that preceded it.

In August 2006, Japanese researcher Shinya Yamanaka, MD, PhD, published a paper in *Cell* chronicling his lab's successful transformation of skin cells into "induced pluripotent stem cells," or iPS cells — stem cells that are capable of multiplying in a dish and, almost magically, of potentially differentiating into all the 200-plus types of cells in the body.

Cut to three years later. Pasca, new to the United States, began a postdoctoral fellowship in the lab of Ricardo Dolmetsch, PhD, then an assistant professor of neurobiology at Stanford Medicine. Starting with iPS cells, Dolmetsch and Pasca generated neurons derived from the skin cells of a young patient with a rare genetic disorder called Timothy syndrome, which predisposes people to autism, epilepsy and cardiac dysfunction. Plated on plastic in a lab dish, the neurons grew in culture and could be used to study the disorder on a patient-by-patient basis, without a brain biopsy. The researchers published these results in a cover article in *Nature Medicine* in 2011.

But the neurons were a little sickly, didn't populate the dish very thickly and died too quickly to form working circuits. However, in a breakthrough experiment in 2011, Pasca found that suspending stem cells in a lab flask could induce them to multiply and form spherical clumps. He could then guide those clumps of neurons to differentiate and recapitulate, in three dimensions, some of the features of the brain's outermost and most evolutionarily advanced section: the cerebral cortex.

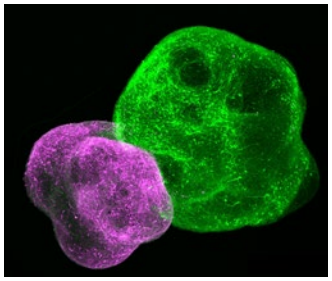
These organoids — small masses, maybe one-sixth of an inch across and composed of about a million cells — thrive in culture, some of them for more than 800 days. That's long enough for some very slow-maturing brain cells to make their appearance, enabling researchers to see things happen that would have been missed using any other experimental system.

For example, some brain cell types spontaneously undergo significant functional changes at around 280 days or so of culture. If that number strikes you as nonrandom, you're onto something. It marks the time from a child's conception to birth. "It's as if they've got a clock," Pasca said.

In 2015, Pasca (who by then had his own lab at Stanford Medicine) and his group published a paper in *Nature Methods* describing the transformation of iPS cells into neural organoids. Floating in laboratory glassware, these balls consisted of cells that were organized in much the same way cells are organized in the cerebral cortex.

IN A 2017 PAPER IN *Nature*, Pasca's team showed how they'd created two distinct types of organoids — one representing the cortex, the other the subpallium, which is a brain region beneath the cortex that plays a significant role in fetal development and then more or less fades away. The researchers let the two organoids cuddle in the same dish, gave them time to fuse together (they do this on their own!), and watched a set of nerve cells called interneurons migrate from the subpallium into the adjacent cortex. There, the interneurons made contact with the very neurons they're fated to meet in real-world fetal development and set up shop, sprouting branching brush-like tails so they could receive input from one another and forming working relationships with neurons in the cortex to generate neural circuits.

Thus began the era of the assembloid: the union of two organoids to permit the observation of different parts of the brain (or, indeed, other organs) connecting and communicating as they do in a living nervous system. Pasca began to use assembloids as workhorses for learning more about the brain.

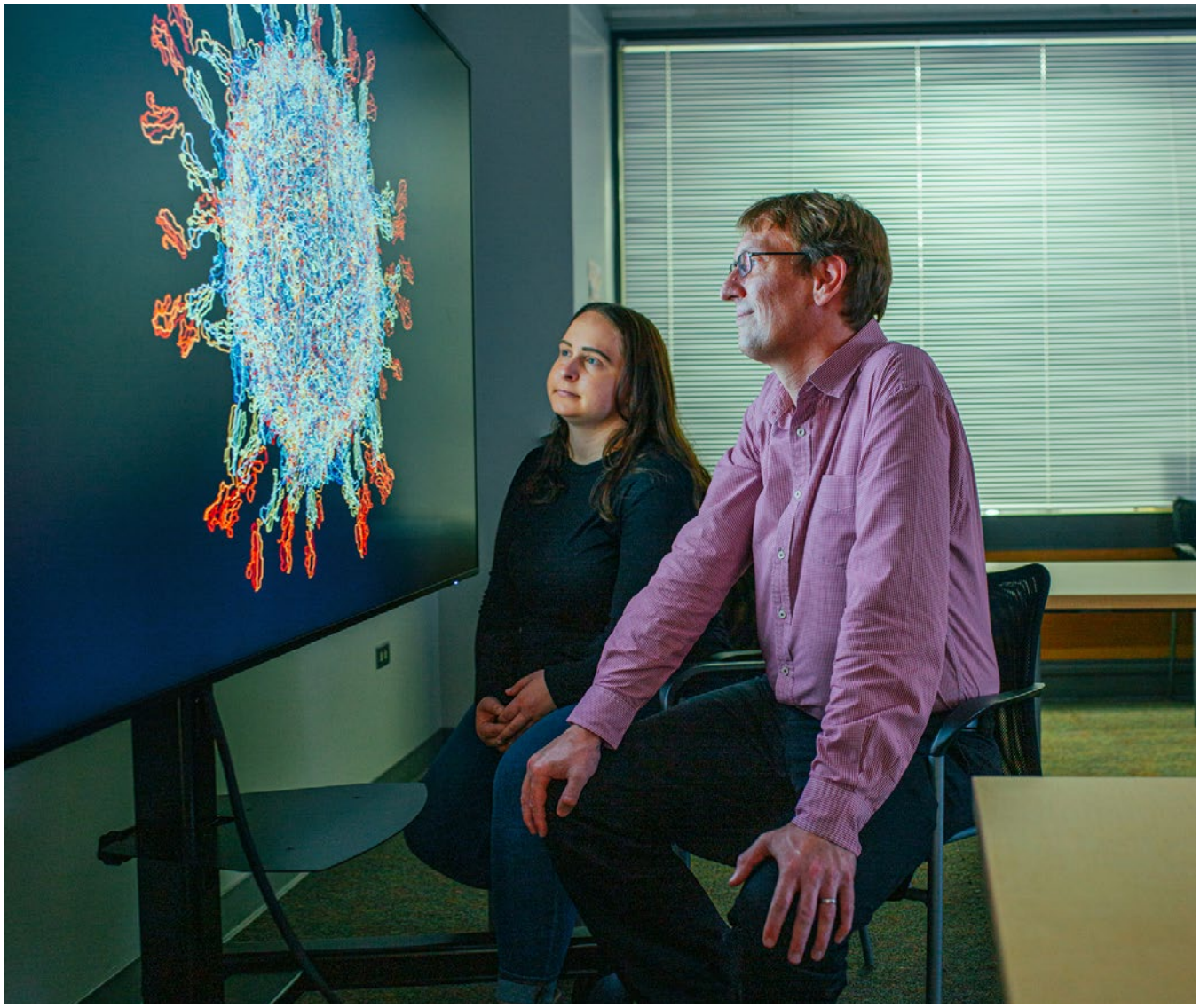


ASSEMBLOIDS FORM WHEN ORGANOIDS UNITE, GIVING SCIENTISTS INSIGHT INTO HOW VARIOUS BRAIN PARTS COMMUNICATE WITH EACH OTHER AND OTHER ORGANS. SHOWN HERE IS A CORTICO-STRIATAL ASSEMBLOID.

Y. MIURA, SERGIU PASCA'S LAB, STANFORD UNIVERSITY.

In the *Nature* paper, Pasca's team showed that interneurons' migration to the cortex proceeds in discrete, stuttering jumps. But in assembloids derived from Timothy syndrome patients, the jumping

ELECTROPHYSIOLOGICAL METHODS
DISORDERS THAT ARISE DURING EARLY DEVELOPMENT



WORKING IN THE LAB OF VIROLOGIST JAN CARETTE (RIGHT), GRADUATE STUDENT CHRISTINE PETERS LEADS A PROJECT USING ASSEMBLOIDS TO STUDY RELATIVES OF POLIOVIRUS THAT CAUSE PARALYSIS. ON THE SCREEN: A POLIOVIRUS AND ITS RECEPTOR.

of interneurons originating from the subpallium is impaired — they jump more often, but the jumps are shorter — with the net result being that they fail to integrate into the appropriate circuitry in the cortex.

By 2020 he'd shown, in a paper published in *Cell*, how to string together three organoids — representing skeletal-muscle tissue, the spinal cord and the region of the cerebral cortex that's responsible for voluntary movement — into an assembloid mimicking the neuromuscular apparatus that controls every voluntary move we make. Stimulating the cortex organoid on one end of the three-part assembloid caused the muscle mass at the other end to twitch.

PARALYSIS IN A DISH

"WHEN SERGIU SHOWED US HIS RESULTS, we were really blown off our chairs," said Jan Carette, PhD, associate professor of microbiology and immunology, whose team is collaborating with Pasca's group to unravel the pathology of crippling viruses similar to the one that causes polio.

Carette is a virologist who focuses on enteroviruses, a

SERGIU PASCA IS EXPERIMENTING WITH A FOUR-ORGANOID VERSION THAT STARTS BY HOOKING THE STRIATUM, WHICH PLAYS KEY ROLES IN

ANCA PASCA USES ASSEMBLOIDS TO STUDY HOW TOO LITTLE OXYGEN HARMS DEVELOPING BRAINS — A PARTICULAR PROBLEM FOR PREMATURE BABIES.

group of viruses so named because they often infect the intestine ("entero" is the Latin root of the term "entrails") — typically without causing much trouble. It's when these viruses break out and hole up in other thoroughfares and orifices that symptoms develop.

On rare occasions, enteroviruses get into the nervous system. Then symptoms can be severe. The once-dreaded poliovirus, responsible for the crippling childhood disease that is polio, is an enterovirus. Fortunately, we don't see many cases of polio anymore — it's been all but eradicated globally, thanks to effective vaccination drives.

But a couple of poliovirus's close cousins — notably one called EV-D68 — worry those who pay attention to emerging diseases. Unlike most enteroviruses, EV-D68 doesn't replicate well in the intestinal tract. Instead, it's well adapted to our upper respiratory tracts. Like other respiratory viruses, it's transmitted by coughing, sneezing and sometimes even by simply breathing. If it infects the spinal cord, EV-D68 can cause a paralytic syndrome called acute flaccid myelitis.

Cases of the syndrome are still rare but have recurred in

THE CORTEX TO A BRAIN REGION CALLED BOTH PHYSICAL MOVEMENT AND PSYCHOLOGICAL MOTIVATION, THE TWO DRIVING FORCES OF MOTION.

alternate years with increasing frequency since 2014. (An exception is 2020 — cases dipped, probably a result of COVID-19-pandemic-induced social distancing, Carette said.) In all, there have been about 700 cases of confirmed acute flaccid myelitis in the United States since August 2014, when the Centers for Disease Control and Prevention began tracking them.

"We're interested in understanding how the virus causes paralysis," Carette said. "Somehow, the virus reaches the spinal cord, invades motor neurons and damages them so they can't direct normal muscle movement. We're asking how, exactly, these viruses interact with human cells. Which cells do they infect? What receptor or receptors on those cells do they hook up with in order to do this? How do they then cause damage to these cells? And how does the immune system respond?"

Questions like those are more easily asked than answered. EV-D68 is tough to study because mice, the workhorses of biomedical research, make lousy models for enteroviral disease. Like poliovirus, EV-D68 naturally infects only primates. That's great in the sense that, in the absence of nonhuman reservoirs, it may be possible in principle to eradicate the virus by vaccinating everybody, should a vaccine be developed.

But from a researcher's standpoint, it's a challenge. And not the only one. "Viral replication seems to happen mainly in the spinal cord," Carette said. "That's a difficult place to get tissue from."

So Carette is collaborating with Pasca, using the latter's assembloid construct to induce what one could call "paralysis in a dish." "We're looking hard at the spinal-cord/muscle junction, where infection probably occurs," Carette said. "There's a variety of neuronal cell types in the spinal cord. Nobody knows, yet, which of them is the virus's target."

By introducing the virus to spinal-cord organoids or spinal-cord/skeletal-muscle assembloids, then inspecting individual cells to see which ones now contain EV-D68 genetic material revealing the virus's presence, the scientists hope to determine which types of neurons are successfully penetrated by EV-D68. They also want to learn how the virus gets in.

Monitoring the activation levels of essentially all the genes inside an infected neuron may reveal which genes' activity levels jump or shrink most markedly in response to infection. That will provide clues to how infected cells attempt to stave off viral replication and to summon assistance from the immune system.

Carette also hopes to use assembloids to compare the virulence of EV-D68 strains from 2014 with those from later years to see if the virus is getting more virulent and, if so, why.

"We're very excited about this project," he said. "The options are limitless."

THE DEVELOPING BRAIN

Anca Pasca, MD, an assistant professor of pediatrics, was a key, hands-on co-author of Sergiu Pasca's 2015 *Nature Methods* study describing the first generation of cortex-mimicking organoids. Now she's focusing on the effects of hypoxia — too little oxygen — on the developing fetal brain, exploring its effects on the interneurons that migrate to the cerebral cortex from the fetal subpallium during pregnancy. Once inside the cortex, these interneurons form complex circuits with resident excitatory neurons, shaping the bursts of activity those neurons produce and discouraging bouts of frenzied firing that characterize neurodevelopmental disorders from epilepsy to autism to schizophrenia.

A deficit of interneuron hookups puts the developing brain at risk for these conditions, Anca Pasca said. And autopsies have revealed that the cerebral cortex of a prematurely delivered baby contains reduced numbers of interneurons compared with that of a full-term newborn.

"We thought this might be related in part to hypoxia," she said. "Even before birth, fetuses that have been deprived of oxygen due to congenital heart disease have smaller brains. And prematurely born babies' lungs are immature. We have to breathe for them, which we do by applying oxygen-supplying masks to their noses or putting breathing tubes into their windpipes. But because their lungs are undeveloped, the oxygen doesn't easily pass from the lungs into the blood."

Is hypoxia killing interneurons before they can embark on their journey to the cortex? Preventing that migration from getting underway? Slowing it to a crawl during a critical

period, so the interneurons never quite reach their destination? Or preventing hookups once the interneurons arrive?

To find out, Pasca's been working with a cortex/subpallium assembloid, like the ones Sergiu Pasca's team developed in 2017. By exposing these assembloids to hypoxia, she has shown that one of the major problems caused by hypoxic conditions lies in impaired migration of interneurons on their way to the cortex.

"They get stuck on the road," she said. "They don't migrate as well. So, fewer of them later integrate into the circuitry in the cortex."

Her team has identified a key substance, produced naturally in the brain, that counters this impairment and that, used as a supplement, could possibly ameliorate the damage induced by hypoxia.

"We can see that the cells are already actually trying to make this protein," Pasca said. "Can we help them out by giving them more of it?" She hopes to move rapidly into experiments with living nonhuman primates to clear the path for a clinical trial.

WHAT COMES NEXT?

IN THE CARDS ARE **more-ambitious assembloids. Sergiu Pasca is experimenting with a four-organoid version that starts by hooking the cortex to a brain region** called the striatum, which plays key roles in both physical movement and psychological motivation, the two driving forces of motion. That opens the door to more-intensive study of movement and psychiatric disorders. Pasca and his colleagues published the entire recipe for generating cortex/striatum assembloids from scratch in *Nature Biotechnology* in 2020. He also intends to fold in another brain region called the thalamus, which feeds sensory input from the outside world to the cortex.

"I don't think we've reached the limits of complexity yet, but we will," he said. "We can in principle put four, five, maybe six organoids together before it becomes too challenging for nutrients and growth factors in the culture broth to reach cells deep within these multi-cellular structures." In any case, organoids in a dish don't contain every cell type resident in the tissues they mimic. They lack blood vessels, for example, whose absence means an assembloid can absorb oxygen and nutrients only at its surface. That's growth-limiting.

One way to get around such limitations is through transplantation. Pasca's team has been grafting human cortex organoids into the brains of living laboratory rats. Preliminary indications are that the human brain tissue not only engrafts into the rats' brains but also receives sensory input and becomes permeated by blood vessels supplying oxygen and nutrients and carting away metabolic waste, in turn permitting the engrafted circuitry to keep growing larger, better integrated, more complex …

And … more human?

That's a good question, said Pasca. As are these: What animals should be considered off-limits for human brain-tissue implants? (All primates, Pasca answers.) Is it acceptable to provide sensory input to a human assembloid, whether in a dish or in a living animal? What about introducing a pain pathway?

Cutting to the chase: Could assembloids ever acquire anything approaching consciousness? The short answer is that it's a long way off, and it may never be possible. Still, this is an example of how Pasca's work defines new frontiers of biomedical ethics as it helps define the foundations of neurodevelopment.

Careful about crossing any ethical lines, Pasca has been proactive all along, consulting at every step of the way with Stanford bioethicists; participating in the National Institutes of Health–sponsored Brainstorm Project, designed to develop ethical guidelines for organoid- and assembloid-associated research; and engaging in discussions with a committee convened by the National Academies of Science, Engineering and Medicine to probe the ethics of neural organoids, transplants and mixed-species experimentation.

Meanwhile, the rats into whose brains Pasca and his colleagues have transplanted lab-grown human cortex organoids don't seem to be acting like anything but the rats they still are.

"We put them through a battery of behavioral tests but found no discernible movement or memory deficits or brain-wave abnormalities," Pasca said. Nor did the scientists observe any signs of *enhanced* performance in these murine organoid-recipients.

There's always been a market for depicting the future as a scary scenario. But nothing like a living brain inhabiting a dish is in the forecast. Meanwhile, back in the here and now, the real danger may lie not in assembloid research's moving too quickly, but in its proceeding too slowly. First and foremost, Pasca told his TED audience, "an assembloid is not a brain — it's just a working model of some of the circuitry resident in a living, functioning human brain."

And second, he told them, "1 in 5 people have some kind of psychiatric illness. But the brain is hard to study, and the success rate for finding new drugs for brain disorders is lower than for any other organ — largely because, until now, we couldn't study live human brain tissue from patients."

Now we can.

At the end of his talk, Pasca got a standing ovation. **SM**

— Contact Bruce Goldman at goldmanb@stanford.edu

WEB EXTRA:
How are assembloids created? Video with more details:
stan.md/AssembloidScience

FEATURE

Imaging blows up

CONTINUED FROM PAGE 19

This is the guiding principle in Skiniotis' lab as he and his team pursue the next generation of molecules that bind to receptors that trip off a desired cellular response. They're involved in designing these receptor-binding molecules, known as ligands, for three receptor proteins: the opioid receptor, the serotonin receptor and the cannabinoid receptor. Why? Each one has huge potential in treating or assuaging symptoms of human disease, but each also has drawbacks.

"Opioids are great analgesics, but they have severe side effects, the most serious of which is suppression of breathing, which is why people die when they overdose," said Skiniotis.

But what if the opioid receptor could be targeted to trigger only the pain-mitigating effect, while the molecular pathway that compromises breathing remained silent? That's why structural specificity and understanding mechanisms — down to the atom — is so crucial for drug design.

"We're trying to make brand new compounds that elicit only desired effects for human health," Skiniotis said.

That same premise applies to his research on the serotonin receptor. Studies have shown that micro doses of the drug lysergic acid diethylamide, or LSD, which targets the serotonin receptor, can effectively treat post-traumatic stress disorder.

"So the question is, can we identify ligands that are good at treating post-traumatic stress disorder by hitting the serotonin receptor without triggering hallucinogenic properties?"

Similarly, he asks whether it might be possible to harness the structural nuance of the cannabinoid receptor to selectively activate the pain-dampening properties without the person feeling intoxicated?

"I cannot tell you, 'Yes, we have new drugs that will work for sure,' or even that this idea will be a success in the near future," said Skiniotis. "But I can tell you that we have new promising and highly specific ligands that we're testing and that we're using cryo-EM to continue to finesse the structures of these molecules. Through these ligands, we have started exploring the basic properties of biology so that, perhaps, one day, there will be new drugs that can make a difference." SM

— Contact Hanae Armitage at harmitag@stanford.edu

FEATURE

Vicious circles

CONTINUED FROM PAGE 29

They had only studied cells from glioblastomas — what if the phenomenon were specific to only that cell type?

To find out, the researchers obtained tissue samples from 17 different types of human cancer, including brain, breast, colon, lung and blood cancers. They found that nearly half of all human cancers contain ecDNA, and that these circles frequently carried multiple copies of cancer-driving oncogenes. The number of circles in each cancer cell varied widely, but they were rarely found in healthy tissue. They published their results in 2017, shortly before the seminar at Stanford.

"We believe that ecDNA is responsible for resistance to so many of our current cancer therapies," Mischel said. "These circles are driving the production of massive amounts of cancer-promoting proteins."

CHROMOSOME CONUNDRUM

ABOUT THE SAME TIME that Mischel and his colleagues were peering into the nuclei of glioblastoma cells, Chang was grappling with his own mystery as he tried to learn how the three-dimensional structure of chromosomes affects gene expression and cell growth in cancer cells.

Normally tightly wound (remember those excited kindergartners?), the section of the chromosome containing a gene to be expressed must be unpacked so it can be accessed by the relatively bulky protein machines that trundle across genes to copy their sequences into mRNA.

Chang and Stanford colleague and professor of genetics Will Greenleaf, PhD, developed a way to generate a global "accessibility profile" to identify regions of active gene expression. There was just one problem: The data was indicating there were far too many accessible regions around oncogenes in cancer cells than could be explained by the numbers of known gene switches on chromosomes.

"Most human DNA is not easily accessible," Chang said. "But these oncogenes were generating such dominant signals in our assays that we had to set them aside to analyze later. It turns out that these oncogenes were on ecDNA."

He brought up the problem to Mischel after the seminar.

"When Howard showed me the data, I almost fell out of my chair," Mischel said. Working together, the researchers began to churn out research papers that outlined exactly how the ecDNA circles helped cancer cells thrive.

In 2019, Chang and Mischel published a paper in *Nature* showing that the oncogenes on the ecDNA are much more active than their chromosomal counterparts.

The researchers soon learned that the shape of the ecDNA molecules was key. "Think about the architecture of a circle," Mischel said. "On a chromosome, control elements are usually located near the genes they control. But when you take that linear structure and make it into a circle, everything is that much closer. It fundamentally changes how gene regulation happens."

Once again, location is important.

"Most of the time we see all the ecDNAs in a cancer cell congregating in a single spot in the nucleus," Chang said.

"We found they form a new structure we called a hub in the nucleus, with multiple oncogenes on tens or hundreds of circles. The regulatory regions on the circles talk in a promiscuous way with other circles, so that switches from completely different chromosomes can affect the expression of genes to which they would never normally have access."

Think of the Red Rover analogy. It's possible for whispered instructions to be shared between one child and another further down in the line by creating a loop in the kid "chromosome." But it's much more difficult for a child in one chain to get close enough to communicate that same secret message to another in the opposite lineup across the field. Now imagine the same hand-holding children playing Ring Around the Rosie, spinning and jumping in multiple small groups on a shared playground. It's child's play (literally!) for participants in different circles to bump into one another and share confidences.

"This is a really surprising result," Chang said. "These circles are acting as a collective in ways that biologists have never seen before. The dogma that regulatory regions act only on nearby genes on the same chromosome simply ceases to exist."

Further research by Chang and Mischel showed that blocking the formation of the protein complex that glues the ecDNAs together causes the circles to fly apart and stops the expression of the oncogenes. In December 2021, they published a bombshell paper in *Nature* detailing their results and speculating that drugs targeting the hub complex could usher in a new class of therapy for many kinds of cancers.

FULL CIRCLE

THE PROSPECT OF ELIMINATING the oncogene expression of all the ecDNAs in a cancer cell in one fell swoop is tantalizing. Chang and Mischel are among several co-founders of a company, Boundless Bio Inc., focused on developing ecDNA-based therapies for patients with intractable cancers.

"I'm most excited about the concept of new therapeutics," Chang said. "Cancer is a disease of genes. Until now we've focused on developing a bespoke solution for each cancer type. If there's an abnormal enzyme, we try to block that enzyme. But if we can disrupt the hub that binds these circles together, we can potentially treat many types of cancer with one approach."

Stopping them from forming at all is another possible option. But many questions remain.

"There is so much more we want to learn about ecDNA," Mischel said. "How do they form, and how are they maintained? Is it possible to stop them from developing? How does the hub work to hold the circles together? And finally, how do they escape the immune system?"

The continuous, rapid selection ecDNAs undergo after each cell division also serves as a built-in research tool to identify which oncogenes are most important in specific types of cancer. "If an ecDNA provides no growth advantage, it would be lost from the cell population right away," Chang said. "Thus, ecDNAs in cancers tell scientists and physicians exactly what is fueling cell growth."

Fundamental changes aren't always easy, or popular. But Mischel and Chang are confident that their findings will lead to a sea change in our understanding of cancer and how to treat it.

"People are visual learners," Mischel said. "But the molecular world is too small for us to see. So we make maps to help us understand. In cancer genetics, the map has been centered on the role of chromosomes. But these findings have upended this map. Now we are redrawing our map and recharting our course to help patients with the most aggressive forms of cancer." SM

— Contact Krista Conger at kristac@stanford.edu

FEATURE

A guide through the cancer labyrinth

CONTINUED FROM PAGE 45

But mostly, she let the women talk freely.

The women's stories echoed the experiences of the steering committee members. "From a cultural perspective, we don't talk about cancer in our families, though I think that's changing," said Tuttle, who has two close family members who have had breast cancer. "With the older African American women, it's something they don't talk about because there is this shame in having people know you have it. But it's to their detriment, because family members can reach out and be supportive, find resources and accompany them on doctor visits. When you're not a patient, you hear things others don't hear and you ask questions."

Taylor Hollis, the youngest steering committee member at 28, said Black women might be reluctant to ask questions of practitioners that could be important in their decision-making and to advocate for themselves in a health care setting.

"I think there's a trait of just following authority and not questioning things. I think that runs in African American communities," said Hollis, who had a close family member with breast cancer and who recently had her own breast cancer scare. "It's not wanting to know if it's bad or not wanting to appear noncompliant."

She said a general mistrust in the Black community of the medical establishment might discourage some women from seeking early care, before the cancer progresses to a serious stage. Moreover, Black women might be uncomfortable sharing their feelings and concerns about the disease with a white practitioner, Tuttle said.

"I think it's a problem when you have practitioners who don't look like them, can't understand them and don't encourage them to be more upfront about their personal feelings about being diagnosed," she said. "They should specifically ask for a provider of color. But what's most important is to have practitioners who have empathy and listen to them."

Gay said that a recurring theme among Black women regarding health care is the feeling that doctors don't take their concerns seriously. Three years ago, for example, she discovered a lump in her breast and had a milky discharge. The doctor, who was not affiliated with Stanford Medicine, told her "it was probably nothing," but Gay pressed for testing. The physician ordered a diagnostic ultrasound but never sent her the results.

"No one ever followed up to let me know what was going on. What if this was something really serious?" she asked.

Several months later, she changed jobs and health plans and went for a second ultrasound and mammogram, which confirmed the presence of a mass in her breast, which clinicians are now monitoring. She felt that doctors generally minimized her concerns until she came to Stanford and finally got some answers.

Previously, doctors had told her: "'Well, you're young, so we're not too worried about it.' But I'm worried out of my mind," she said.

In the interviews with participants in the Stanford Medicine peer navigation study, researchers found common threads, some of which surprised Rosas. For example, she presumed the women would primarily be concerned with practical matters like transportation and child care. Instead, many participants prioritized access to nonmedical alternatives for managing the disease.

Tuttle said one of the women she spoke to opted to use herbal medicines, nutrition and other alternatives in lieu of chemotherapy to avoid the toxic effects of treatment. Another declined a second round of chemotherapy because of concerns about side effects.

In general, the women expressed strong interest in approaches that promote general well-being, such as massage, gentle exercise and mindfulness practices to reduce stress. They also emphasized the importance of faith in their cancer journeys.

"It is their own spiritual belief and strength that helped pull them through — their ability to be supported by church groups that may have a health ministry where they can talk to other women, a prayer group or a consultation with their minister," Tuttle said.

Waugh, who was an informal peer navigator for years, said that before the COVID-19 pandemic, she created a "spiritual spa" for women at her church in Hayward who had breast cancer. It included a space with meditative music, lavender oil and tea, giving the women a chance to share whatever was on their minds.

"In the faith-based community, we pray about these things," she said. "If there is a lump, you pray about it and believe God is going to remove it. So what happens if he does not remove that lump? What is the next step? Once you get them into a comfort zone to understand that God heals us any way he wants, you can help them understand it's OK to turn to the doctors to help you."

She said it's not what happens in the clinic alone but also what occurs outside the clinic that can make a difference in how a person feels and whether they're likely to do well.

"Once you walk away from treatment, that's where the work really begins," said Waugh. "That's when you need to have somebody who has empathy and deep-rooted compassion. Unless you've walked that journey, you don't know what somebody else is going through. And when you share your experience, it makes that person's journey a bit lighter." **SM**

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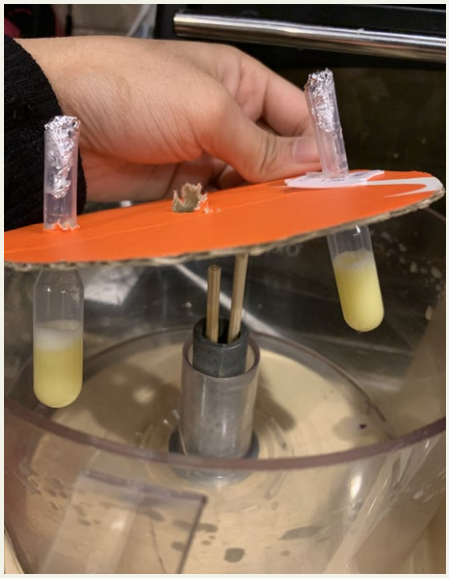
TRY THIS AT HOME

STUDENT RESEARCHERS TURNED TO EVERYDAY ITEMS TO ISOLATE ANTIBODIES FROM CHICKEN EGGS

In the fall of 2020, Stanford freshman Caitlin Kunchur was living with her parents in South Carolina, experiencing college life through a steady stream of video meetings. More than 2,500 miles from Stanford's main campus in Palo Alto, California, her chances to work in a university research lab were nearly nonexistent. Then a post appeared on one of her Slack channels — a shared community for students interested in biodesign. Protein chemist Daria Mochly-Rosen, PhD, wanted help devising a cheap and easy way to isolate antibodies from the yolks of chicken eggs.

Mochly-Rosen had been working with a team of scientists — through the Stanford SPARK Global Program in Translational Research that she founded and leads — to show that chickens vaccinated against COVID-19 lay eggs containing virus-fighting antibodies. Researchers had learned that these IgY antibodies, isolated from egg yolks, could be used as nasal drops in humans to provide short-lived but effective protection against viruses.



Students used a homemade centrifuge created out of a food processor, cardboard and skewers to isolate antibodies from the yolks of chicken eggs.

Typically, researchers isolate IgY from eggs using specialized reagents and expensive centrifuges — which spin tubes at high speed to separate molecules by their densities. Mochly-Rosen wanted something much more accessible. "I thought if we could just figure out a way for people to isolate these antibodies using common household supplies, this could be a really great solution for low-income countries to be able to generate some protection against COVID-19 or future viruses," she said.

Kunchur, four other undergraduates and two master's students, responded to Mochly-Rosen's query. "I was stuck at home with a lot of free time, and getting to actively participate in COVID-19 research was not an opportunity I could pass up," said Kunchur.

Some team members tackled how to separate and filter parts of the egg and alter the chemical properties of the yolk (settling on white vinegar, baking soda and salt as key ingredients), while Kunchur designed a cheap centrifuge to separate out antibodies.

"I literally wandered around my kitchen and garage looking at everything I could find that had spinning parts or motors," she said. Spinning a wheel didn't generate enough force. A blender didn't easily hold the samples. But an old food processor sparked Kunchur's imagination. She dropped some skewers into the central spindle of the food processer, jammed a cardboard disk on the top and pushed tubes of egg yolk into holes in the cardboard.

Kunchur filmed the test run of the improvised centrifuge, then played it, in slow-motion, for the team during a late-night video conference. "It was really an 'aha!' moment," she recalled. "We could see the separation as it happened and got a beautiful antibody pellet at the bottom of each tube."

Eventually, the students designed a 3-D printed version of the tube-holder to convert a basic $44 Hamilton Beach food processor into a centrifuge. They verified that they were isolating IgY antibodies and calculated that, using their protocol — even including the cost of a food processor — anyone can isolate chicken antibodies for about 66 cents a dose. That is about one-eighth the cost of IgY production using standard methods, they reported in the *Journal of Global Health* earlier this year. With their publication and an early-phase safety and efficacy study of anti-SARS-CoV-2 IgY antibodies, they hope that entrepreneurs or nonprofit health groups pick up where they left off, producing and distributing such kits for the local isolation of antibodies.

"These students were all incredibly creative and motivated," said Mochly-Rosen, the George D. Smith Professor of Translational Medicine. "I think it's pretty amazing what they were able to do even while stuck at home."

BY SARAH C.P. WILLIAMS

PHOTO COURTESY OF CAITLIN KUNCHER

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Ready, set, genome

RECORD-SETTING DNA SEQUENCING FINDS ANSWERS FOR PATIENTS FAST

Thanks to a new, ultra-rapid technique devised in the lab of Stanford Medicine geneticist Euan Ashley, MD, PhD, scientists have cut down the time it takes to sequence a human genome from several weeks to 5 hours and 2 minutes, a feat that earned the research team a rare accolade in medicine: a Guinness World Record.

"It was just one of those amazing moments where the right people suddenly came together to achieve something amazing," Ashley said. "It really felt like we were approaching a new frontier."

Genome sequencing provides a window into a person's molecular makeup — a DNA blueprint of nearly everything in that person from hair color to disease risk. For people who have diseases that stem from a genetic variant, DNA sequencing can reveal that, helping scientists determine precise diagnoses and treatments.

"A few weeks is what most clinicians call 'rapid' when it comes to sequencing a patient's genome and returning results," said Ashley, the Roger and Joelle Burnell Professor in Genomics and Precision Health. "Genetic tests just aren't thought of as tests that come back quickly. But we're changing that perception."

The team was able to use the new rapid sequencing approach, which simultaneously harnesses the power of multiple genome sequencers, in a clinical trial with 12 patients, five of whom were diagnosed with the help of the sequencing information.

In one case, a 13-year-old boy, Matthew Kunzman, arrived at Stanford Hospital in rapid decline; his heart was failing. Using the new technique, it took Ashley and his team a matter of hours to reveal that genetics were to blame for Matthew's condition, myocarditis — inflammation of the heart. The only solution would be a heart transplant.

Matthew was immediately put on the transplant list and received a heart within a month. A year later, his mom said he's doing "exceptionally well."

Now, Ashley and his collaborators are optimizing their technique to speed up sequencing and diagnosis even more. "I think we can halve it," Ashley said. "If we're able to do that, we're talking about being able to get an answer before the end of a hospital ward round. That's a dramatic jump."

— BY HANAE ARMITAGE

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