Berkeley College of Letters & Science

SPRING 2022

DIVISION OF BIOLOGICAL SCIENCES

Letter from the Dean

Dear Friend,

We in the UC Berkeley community have lived through a lot during this past year. Days with poor air and an ominously orange sky from so many wildfires. Persistent drought. Persistent COVID-19 pandemic.

What also persists, however, is our determination that Berkeley won't just endure current and future challenges but will emerge stronger than ever. We must draw on our reserves of hope and optimism and continue to emphasize, for everyone on campus, the importance of wellness.

The pandemic exacerbated a preexisting spike in depression and anxiety disorders worldwide, especially among college-age individuals. We know that mental health disorders contribute to a range of other health problems. By encouraging wellness — the process by which we can attain or maintain physical and mental health — we will foster our collective creativity and empathy — making for a better campus environment, and a better world.

I worked with Steve Murray and Dacher Keltner to develop an undergraduate minor focused on the interdisciplinary science of health and wellness, which launched in the fall (see the story on page 11). This curriculum draws on expertise from several areas in the College of Letters & Science, including research by the Greater Good Science Center.

My division has also partnered with the School of Public Health, the College of Chemistry, and the Division of Computing, Data Science, and Society on three strategic interdisciplinary faculty recruitments. The first position, aptly enough, will be in the field of emerging zoonotic diseases, supporting a new research initiative around future pandemic readiness (see the story opposite). The second position will collaborate with chemists to pursue immunological breakthroughs. The third faculty member will advance data-driven biomedicine by applying artificial intelligence methods to cancer research and personalized treatment.

Bringing fresh scientific expertise to Berkeley is essential to maintain our academic strength — our intellectual wellness. The size of the faculty has remained steady for more than a decade, but the number of students has increased tremendously. Partnering with other campus units enables us to bring in scholars who are breaking down disciplinary silos by pushing the boundaries of their fields. This can only serve to recharge the wellspring of talented and curious students, faculty, and researchers whose work drives the Division of Biological Sciences.

I am pleased to share some of this exciting research with you in the following pages. If you would like to learn more, please be in touch with us. Thank you for your ongoing interest and support!

Warmest Regards,

Michael Botchan, Ph.D.
Dean of Biological Sciences

COVER PHOTO: In the Nayak Lab, methane-producing microbes known as archaea are grown for research. The changing color of an added dye, resazurin, reflects the oxygen level in the growth medium. See page 8 for more. Credit: Stephen McNally

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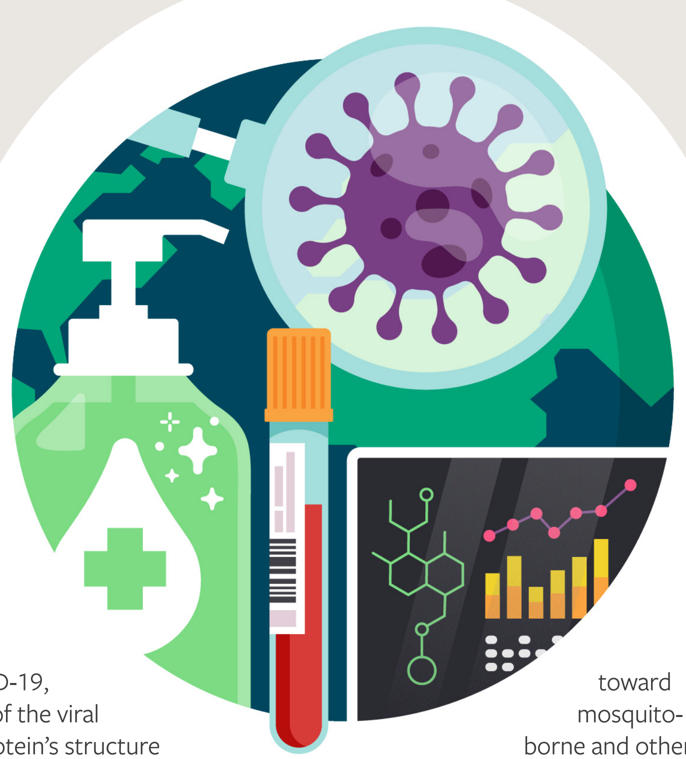
Basic Research in Service of Human Health



As COVID-19 continues its impact on society, it's time to prepare for the eventual next pandemic. That was one clear message from a virtual salon hosted by Chancellor Carol T. Christ in June to highlight Berkeley's interdisciplinary initiative in Health and Basic Discovery.

Co-led by the deans of the Biological Sciences Division and the School of Public Health, the initiative foregrounds pandemic outbreaks and antimicrobial resistance as one of three research pillars, the others being chronic disease and environmental health of climate change. Chancellor Christ called Health and Basic Discovery "one of the most pressing multidisciplinary efforts underway at Berkeley."

Eva Harris and her lab group

The salon featured a conversation between Associate Dean of Biological Sciences Ed Penhoet and two distinguished faculty members: Jennifer Doudna and Eva Harris. Moderator Penhoet made the point that basic science enables the technological breakthroughs to confront or cure disease; in the case of COVID-19, studies of the viral spike protein's structure and of mRNA proved crucial to creating effective vaccines.

Doudna, president of the Innovative Genomics Institute and a 2020 Nobel laureate for her role in developing CRISPR-Cas9 as a genome editing technology, discussed how the versatility of CRISPR — a natural viral defense system that evolved in bacteria — allows it to be applied for many practical purposes, including detecting traces of DNA or RNA for disease diagnosis. Mammoth Biosciences, which Doudna co-founded, has produced a CRISPR-based rapid test for COVID-19 infection.

Doudna said, "How do we ensure that next time — and we know there will be a next time — that we have the ability to quickly program a diagnostic tool to detect a new virus?"

"One of the key aspects of Berkeley, and where Jennifer and I certainly come from, is this very deep and strong belief that science is a public good," said Harris, a professor of infectious diseases and vaccinology. She described her holistic approach toward mosquito-borne and other viruses that spans from basic biological lab research to community public health and capacity building to combat diseases on the ground in Latin America. Harris said, "The best pandemic preparedness is local capacity" to be able to respond to outbreaks in real time.

As for what Berkeley needs to expand its capacity to train leaders ready to face future pandemics, the professors stressed greater graduate student support. Berkeley competes with universities able to offer a full free ride to doctoral students. Grants can be hard to find for students in the early phases of their research, and foreign students are not eligible for federal funding. Doudna expressed hope that the graduate program in her home Department of Molecular & Cell Biology, would be endowed by a generous donor.

Other "wish list" items to enhance Berkeley's prowess in pandemic preparedness include faculty experts on viruses and other infectious diseases, bio-secure laboratory facilities, and a ready fund to facilitate creative collaborations, like those spawned suddenly by COVID-19, when the next pandemic arises.

DELIVERING ON THE PROMISE OF CRISPR CURES

During a press conference after becoming Berkeley's latest Nobel laureate in Chemistry, Jennifer Doudna was asked what technical challenges could impede practical uses for her award-winning discovery, CRISPR-Cas9 genome editing. She quickly identified delivery — the ability to guide engineered molecules to the right cell or tissue type for their intended task — as "a common challenge that we face right now in the field." On another occasion, Doudna has suggested that solving the delivery of gene editing therapies will mark as big an advance as the development of CRISPR-Cas9 itself.

One of Doudna's former postdoctoral researchers, Ross Wilson, is among the Berkeley scientists pursuing this advance as director of therapeutic delivery at the Innovative Genomics Institute (IGI). An assistant adjunct professor of molecular and cell biology, Wilson co-leads the IGI Delivery Collective, a collaborative effort among three Berkeley labs and one at UCSF to develop technology that safely enables the editing of deleterious DNA.

Scientists have devised a few possible ways to deliver gene therapies. One technique places therapeutic molecules inside a viral vector, exploiting the ability of viruses to force entry into cells. Another approach relies on encasing the cell-bound cargo with lipid-based nanoparticles.

Says current Wilson Lab postdoc Dana Foss, "Our lab is dedicated to working on developing an entirely different approach, that is, engineering the Cas9 enzyme itself to be its own delivery agent."

Cas9 is quite adept at binding and cutting a sequence of DNA, but it can't enter a cell or navigate once inside. Like adding new features to a basic Swiss Army knife, Wilson's team of protein engineers wants to imbue Cas9 with these extra functions.

Their preferred approach involves creating customized ribonucleoproteins (RNPs), a combination of RNA and Cas9 protein only 10 nanometers across. An antibody attached to the Cas9 will be recognized by specific markers on a target cell's surface in order to gain entry. Now enveloped by part of the cell membrane, the RNP relies on membrane-disrupting peptides to release it, so the RNP can reach the cell's nucleus and perform editing.

Wilson believes that tiny and transient RNPs are less likely than other delivery methods to produce undesired consequences, such as off-target editing or immune responses. RNPs also lend themselves to scalable manufacturing, which would help make CRISPR cures affordable.

"If you want a gene therapy that goes in, does what it's supposed to do, and then vanishes from the body, RNPs are the best tool in our toolkit," says Wilson. "With some improvements, they may soon represent an all-in-one option."

Ongoing experiments using RNPs to perform targeted edits of human immune system T cells and blood stem cells have been encouraging. A current clinical trial to treat the painful blood ailment sickle cell disease involves extracting the patient's stem cells, editing them in the lab using Cas9 RNPs, and then returning the modified cells to the patient. But the ultimate goal is to be able to deliver CRISPR cures directly to diseased cells or tissues.

When the Brain's Barrier is Breached

As our bodies age, we become more vulnerable to bone breakage and other forms of injury or disease. The same goes for the brain. In particular, a sudden trauma or accumulated "wear and tear" on the brain's built-in defense shield could expose us to a cascade of cognitive negative consequences.

This shield, known as the blood-brain barrier (BBB), comprises a mesh of specialized blood vessels. We think of blood vessels as conduits sending blood throughout the body, but the BBB's vessels offer extra security — filtering blood to permit nutrients to pass while blocking bad things (like toxins and pathogens) from reaching the brain.

Traumatic brain injury can cause leakage from the BBB. So does normal aging. By age 50, 30% of people have some weaknesses in this circulatory shield. After age 70, the percentage of people with some degree of BBB degradation doubles. This matters because substances in the blood entering the brain can trigger inflammation and a sequence of damage that culminates in cognitive decline and dementia.

This is a key conclusion from decades of research by Daniela Kaufer, professor of integrative biology and associate dean of biological sciences. In painstaking studies using mice, Kaufer and colleagues identified a primary suspect that activates brain inflammation: albumin, an abundant blood protein. Infusing a mouse brain with albumin for only a few days was long enough to generate symptoms seen in aged brains. Says Kaufer, "We know that a leaky blood-brain barrier is sufficient to make the young brain look old."

When albumin enters the brain, it stimulates astrocytes, support cells for neurons and synapses, by binding to the cells' receptors for the transforming growth factor beta molecule and initiating an inflammatory response. Inflammation increases as more albumin leaks in, leading to cell death and other dire consequences.

But there's also evidence for hope. Kaufer's research shows that blocking the crucial receptor can reverse the damage and restore the BBB's vascular integrity. A week of treatment with an experimental cancer drug IPW that blocks this receptor successfully restores youthful brain function to mice. Kaufer co-founded pharmaceutical startup Mend Neuroscience, Inc. to develop this therapy for use in humans.

Kaufer and her longtime collaborator, Dalhousie University neuroscientist Alon Friedman, wrote about their barrier-breach theory in the May 2021 issue of *Scientific American*, noting that "the results of our work strongly hint that the aging brain retains a capacity for reshaping and restoring itself…" As to the prospect for future therapies that could reduce — or reverse — the cognitive consequences of a leaking barrier, Kaufer and Friedman concluded, "There is a chance to do a lot of good for a lot of people if we can figure these things out."

Four generous gifts support campaign priorities

Amazing mentors.
A welcoming environment.
A top-flight education.
A beloved academic home.

Berkeley touches people in myriad ways. Inspired by how the university transformed their lives and those of others, four families of eminent scientists and educators recently made exceptional gifts benefiting faculty and students in the Division of Biological Sciences.

The generosity of Timothy Springer ’71 and Chafen Lu, G. Steven and Gail Ph.D. ’71 Martin, Frank Davis Ph.D. ’55, and Ronald and Rebecca Harris-Warrick will help recruit and retain the very best faculty while providing invaluable resources for graduate and undergraduate students to realize their intellectual dreams.

Springer, a Harvard professor, is internationally recognized for his discoveries of molecules in the immune system that fight infection and promote disease — a finding that generated new drugs for treating autoimmune diseases. He and Chafen Lu, his spouse and fellow researcher, have endowed a faculty chair at Berkeley, which was recently awarded to Professor James Hurley.

Originally from Sacramento, Springer transferred to Berkeley as a sophomore from Yale. Cal ignited Springer’s passion for science, and he graduated with top honors in biochemistry. “I just had fabulous teachers at Berkeley,” he says. The late Professor Jack Kirsch was a standout. “I would frequently go to his office hours, and I might be the only person there,” says Springer, recalling how the two discussed science at length. Springer credits Kirsch with coaching him to explore research problems in “incredible molecular detail.”

In creating the Kirsch Springer Chair in Biological Sciences, “I wanted to honor that bond between professor and student,” says Springer, whose accolades include receiving the 2004 Crafoord Prize and the 2019 Canada Gairdner International Award.

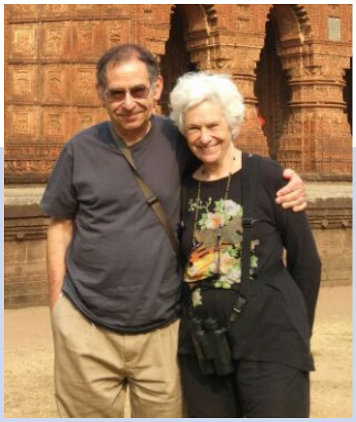
Martin, an emeritus professor of cell and developmental biology at Berkeley, along with his wife, Gail Ph.D. ’71, an emeritus professor at UCSF, also established a faculty chair. The G. Steven and Gail R. Martin Chair will be awarded on a rotating basis to a professor in the divisions of Biological Sciences and Arts and Humanities.

“I had a wonderful career at Berkeley,” says Martin, who arrived as a postdoctoral fellow from England in 1968. Focusing on Rous sarcoma virus, which causes a type of cancer in chickens, Martin identified the viral gene, v-src, responsible for cancerous transformation. His work has shed light on genes that can cause cancer in humans.

Among many campus leadership roles, Martin served as co-chair of the Department of Molecular and Cell Biology, Dean of Biological Sciences, and interim Vice Chancellor for Research.

“What I’ve always liked about Berkeley is that it’s a very supportive and collegial and collaborative environment,” he says. Martin sees his gift as a way to advance “a unique asset,” a renowned public research and teaching institution that gives students of all means access to higher education.

Timothy Springer '71 and Chafen Lu



G. Steven and Gail Ph.D. '71 Martin

Frank Davis Ph.D. '55

Rebecca and Ronald Harris-Warrick

Davis, a Depression-era farm kid from Oregon, relished his graduate studies at Berkeley. "Cal was an epicenter of biochemical research and discovery in the 1950s, and that is exactly what my father wanted," says his daughter, Ann.

Davis became a professor at Rutgers University, where he invented a drug delivery process called PEGylation that coats biological molecules so that they can more easily be used as medicine. His discovery helped pave the way for the biopharmaceutical revolution and is used in the Pfizer and Moderna COVID-19 vaccines.

Honored as the 2013 Alumnus of the Year, Davis "always said Berkeley taught him to think and how to problem solve," his daughter says.

In gratitude, Davis established the Frank F. Davis Endowed Fund for Graduate Fellowships in the Biological Sciences. Upon his death in May 2021 at the age of 100, Davis left a generous bequest to the fellowship fund. Ann Davis says, "He felt that graduate students really needed the financial support to be able to follow their curiosity and their research ideas freely, without having to worry about paying the rent."

Morgan Harris '38, Ph.D. '41 enjoyed a long and happy career at Berkeley, stretching from his undergraduate days well past his retirement as a professor and former chair of the zoology department. A noted experimentalist in cell biology and cancer research, he was a pioneer in understanding the phenomenon of epigenetic inheritance of traits.

Berkeley "was just a very warm, welcoming place for him and supportive of everything he did," says his son, Cornell neuroscientist Ronald Harris-Warrick.

Harris-Warrick and his wife, Rebecca, an emeritus music professor at Cornell, endowed the Morgan and Marjorie R. Harris Scholars Fund honoring Ron's parents, who were both Cal alumni. The scholarships will provide summer lab opportunities for undergraduates aimed at increasing the diversity of students pursuing careers in academic science. "My dad was encouraging of people of all groups," says Harris-Warrick. Berkeley connections run deep on both sides of the family: Rebecca Harris-Warrick's late father, W. Sheridan Warrick, was the longtime executive director of International House.

Manipulating Methanogens Could Help Combat Climate Change

As a graduate student, Dipti Nayak intended to become an engineer, like her father. While studying bioremediation, however, her interest turned to the microbes themselves rather than how they help clean up our messes. She shifted her studies to biology and to the mysterious microbes known as the archaea.

Proposed 45 years ago as a new and distinct kingdom of life, archaea constitute a vast group of microscopic, single-celled organisms that may be among the most ancient living things and possible ancestors to all eukaryotes — that is, to all plants, fungi, animals, and us. Archaea have adapted to live in seemingly harsh environments with extreme heat, salt, or other challenging conditions.

Among the archaea, Nayak focuses on a large group called methanogens. They can be found essentially anywhere with low or no oxygen and possess the unique ability to metabolize methane. “They’re everywhere, but they’re mostly invisible,” says Nayak, an assistant professor in the Department of Molecular and Cell Biology.

Therein lies a problem. Methanogens generate 80 percent of atmospheric methane, respiring it just like we exhale carbon dioxide. Both carbon dioxide and methane are atmospheric greenhouse gases contributing to rising temperatures and climate change. Most attention goes to reducing levels of carbon dioxide, but methane is the next most abundant — and a much more potent — greenhouse gas.

Although it persists for shorter periods in the atmosphere compared to carbon dioxide, methane packs about 80 times more heat-trapping potential. Controlling methane emissions could provide a relatively rapid way to keep global temperatures in check, but instead, says Nayak, “The methane levels are rising and rising fast.”

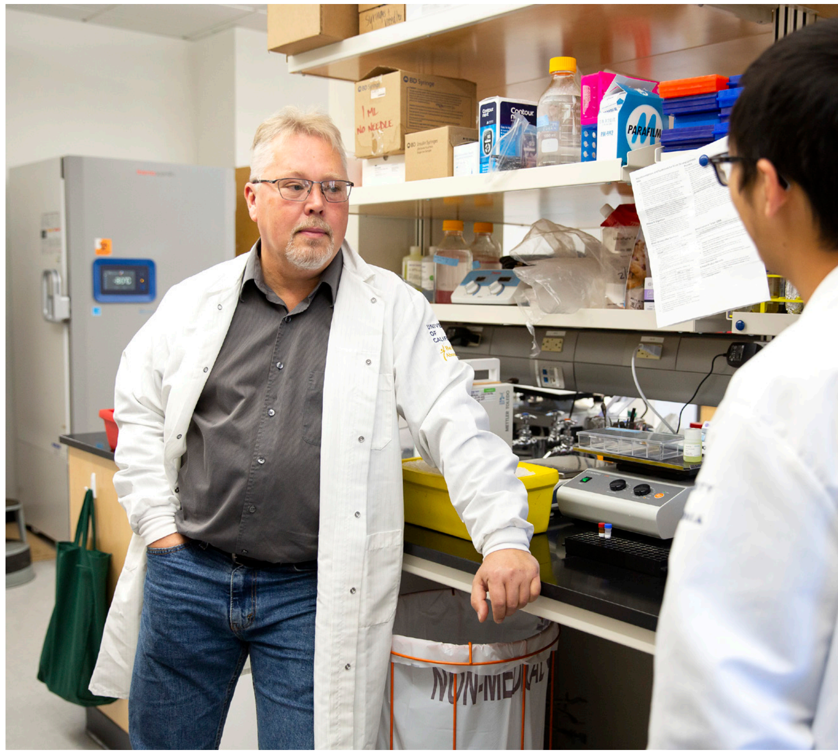
The latest Intergovernmental Panel on Climate Change report stressed the urgency to address methane levels, and methane was on the minds of delegates to the recent COP26 climate conference, where 105 nations pledged to cut methane emissions by 30 percent in this decade. That will entail curtailing emissions from oil and natural gas production, agriculture, rice cultivation, and landfills, among other human sources.

What of those abundant, invisible methanogens? That’s where a biologist like Nayak comes in. She’s investigating whether CRISPR-based genome editing could be used to change the metabolic machinery of methanogens so that their cells can maintain their physiological function without making methane. Ultimately, this could involve engineering the microbial community inside of a cow or in a marsh.

Long before then, Nayak must delve into the basic biological processes that determine where methane comes from and where it goes in the environment. There are other kinds of archaea that can oxidize, or consume, methane, and it turns out that an identical chain of biochemical reactions governs whether methane is produced or consumed. So, with the aid of CRISPR, it may be possible to tease apart the genetic factors that distinguish methane producers and consumers and make the producers less reliant on their problematic product. Summarizing the ambitious goal, Nayak says, “What we hope to understand in our lab is this one single process that’s essentially controlling the net flux of methane on the planet.”

Pursuing Mechanisms Behind Metabolic Diseases

At Berkeley, basic research in cell biology often blends into biomedicine — spawning discoveries with potentially profound implications for our health. Take, for example, the labs of James Olzmann and Anders Näär, and their investigations into the inner workings of human metabolism.



Anders Näär

Olzmann, an associate professor of molecular and cell biology, studies lipid droplets, compartments that store fat molecules for a cell's future energy needs and help prevent cell damage from fatty acids. "For a long time, people thought of them as just these inert fat globules that weren't of much interest for cell biologists," says Olzmann. Now, he adds, "...we're realizing how central they are for lipid biology."

Composed of a distinctive single-layer membrane that surrounds fatty oil and contains regulatory proteins, the droplets influence lipid storage, membrane manufacturing, cell signaling pathways, and other essential processes. Droplet defects have been implicated in obesity, diabetes, atherosclerosis, and fatty liver disease. Understanding how they form and function will contribute vital clues to and, potentially, treatments for these diseases.

"Sometimes you never know where these studies will end up," says Olzmann, "and that's one of the really fun and powerful parts about the systems-level discovery approaches we employ... We often end up in completely new and unexpected territory."

Anders Näär, professor of nutritional sciences and toxicology, landed in unanticipated terrain when his lab shifted to studying microRNAs (miRs), regulatory short genetic sequences that don't code for proteins. Says Näär, "They're emanating from what people call 'the dark matter of the genome' or junk DNA." He's begun to shed light on the connections between microRNAs and metabolic diseases.

For instance, Näär's team discovered miR-128-1 and determined that this microRNA helps regulate levels of circulating cholesterol and fat. Näär describes miR-128-1 as "a metabolic master regulator that was beneficial in ancient times, [that] allowed us to store fat and survive famine, but now it's a nefarious actor in promoting metabolic disease" under our modern conditions of nutritional excess.

Mice fed a high-fat diet for four months also received weekly doses of anti-miR-128, a chemically modified antisense oligonucleotide. This treatment resulted in a 20% decrease in fat and body weight, erasure of Type 2 Diabetes pathology, and elimination of inflammation and excess lipids in the liver. This single microRNA, it appears, may link to the trio of diseases in Metabolic Syndrome — obesity, Type 2 diabetes, and fatty liver disease. Serendipitously, Näär's lab also found that miR-128-1 serves as a therapeutic target in Duchenne muscular dystrophy, a lethal, muscle-wasting ailment of young males.

"We're surmising that there must be other diseases that have prominent roles for microRNAs," he says. "This is just the tip of the iceberg."

Notes from the Field

As soon as summer arrives, Berkeley students depart campus for jobs, internships, or perhaps a well-earned rest. But for graduate students in the Department of Integrative Biology, summer means heading into the field, from tropical rainforests to mountain meadows, and conducting research. We asked a few doctoral candidates to report back on their observations and discoveries.

Kwasi Wrensford

My research focuses on the ecology of two chipmunk species in the Eastern Sierra Nevada of California, the lodgepole and the alpine chipmunk. These species co-occur in significant portions of their range. Over the past century of climate change, the alpine chipmunk's range has shrunk significantly, while the lodgepole chipmunk's range has remained relatively unchanged. I seek to understand how these similar species differ in their capacity to behaviorally adjust to a rapidly changing environment.

I've always been fascinated and deeply inspired by animals. **By exploring nature and learning more about the living things with whom we share the world, I gain a greater sense of belonging and gratitude.** Through my work, I hope to provide another small piece to our understanding of nature.

Valeria Ramírez Castañeda

Understanding biological interactions in the tropics is what inspires me the most. Currently, most knowledge of eco-evolutionary dynamics is based on simple models with few interactions. However, complex interactions occur in highly biodiverse regions in the world. I go to the Amazon rainforest of Colombia to find Neotropical snakes that feed on highly toxic poison dart frogs. These snakes are resisting several toxic molecules, and my job is to identify possible mechanisms of toxin resistance.

Every day in the forest is unexpected. You never know what you are going to find. The rainforest is a fast-changing environment. Once you spend more and more time in *la maniqua*, you realize how full of movement and extraordinarily alive and complex it is.

Nina Sokolov

I study bees, which are crucial for the pollination of wildflowers and crops. I collect bees in alpine wildflower meadows in the Sierra throughout the summer to determine if virus spillover is occurring from managed honeybees into native wild bees. Infectious diseases are one of the reasons behind bee population declines, and I'm passionate about trying to understand how viruses are transmitted between bees in order to conserve healthy populations into the future.

The joy from being out in the field and learning how to see ecological processes unfolding is what inspired me to continue in biology. **Field work allows me to study the natural world intimately, which is pretty much what I want out of life.**

New Minor Cultivates Health of Mind and Body

The experiences of the past two years brought into focus the importance of maintaining physical and emotional well-being. Long before COVID-19 entered our consciousness, the Division of Biological Sciences began laying plans to enable Berkeley students to explore their health in a coherent and concentrated course of study. These plans culminated in October with the launch of the Health and Wellness Minor.

Developed jointly by Physical Education Program Director Steven Murray and Department of Psychology Professor Dacher Keltner, the new minor aims to integrate the biological and social sciences in an examination of happiness, health, and wellness.

Keltner chaired the committee that recruited Murray, who has taught health and wellness for 25 years, and brought him to Berkeley nearly four years ago to expand the Physical Education Program. Murray and Keltner had a similar vision for what the minor could be. "I was excited to come here and work with him to develop the program," says Murray. "We ultimately have the same goal of producing individuals who are happier and healthier and who want society to be happier and healthier."

While similar efforts at other campuses emphasize a physical or policy approach to health, Murray says, "This is one of the first health and wellness programs that truly bridges the psychological and the physiological." It also incorporates analysis of sociocultural factors influencing health and wellness, such as race, class, and gender.

The minor's lower-division requirements include introductory courses on fitness, nutrition, and psychology. Its core curriculum consists of three upper-division courses covering health and wellness from psychological, physiological, and cultural perspectives. One of these is Keltner's perennially popular course on "Human Happiness." A second core course, "Wellness for Life," conceived and taught by Murray, is organized around six dimensions of wellness: physical, occupational, social, spiritual, intellectual, and emotional. The third course, "Health, Wellness, and Culture: A Critical Perspective," will be ready for students starting in the fall. Rounding out the minor's requirements are two upper division electives, selected from more than 80 courses across many disciplines, including "The Anthropology of Food," "The Neurobiology of Stress," and "Sociology of Health and Medicine."

Steven Murray

Murray credits Dean of Biological Sciences Michael Botchan for the sustained enthusiasm and support that were necessary to take the minor from idea to inception. Says Botchan, "It is exciting, especially in these complex times, to pioneer a program that links the domains of health and wellness, enabling one to both examine and internalize the connections between the mind and body."

Now it's up to the students. Considering that some 5,000 students enroll in Berkeley's physical education courses each year, there should be plenty of interested parties pursuing personal health.

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Virtual Lecture Series Continues to Inspire

Spring brings a new season of virtual Basic Science Lights the Way conversations about current research, featuring prominent Berkeley faculty and graduate students. Co-presented by the Divisions of Biological Sciences and Mathematical & Physical Sciences, each event packs into an hour accessible presentations about discovery-driven science, moderated discussion with the speakers, and audience questions. This season's series continues until May 4.

Reflecting Berkeley's breadth of scientific expertise, previous topics in the series have included aging, drug resistance to infectious diseases, cutting-edge biological imaging technology, frontiers of brain research, the pathogenesis of COVID-19, the role of data science in genetics, how nature inspires the design of drugs and robots, and the ecology and evolution of biodiversity.

Missed any past events? Closed-captioned videos of all our conversations can be accessed in the archive at **basicscience.berkeley.edu**.

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