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BIO+



PRINCETON

School of Engineering
and Applied Science





BIOENGINEERING

'The most complex forms in the universe' yield advances in health and engineering

DEAN'S
MESSAGE



EQuad News

Spring 2020
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Few fields of research hold such sweeping promise for improving lives as bioengineering. The ability to understand and manipulate the processes of life is producing new treatments and diagnostics of disease, renewable and efficient sources of energy, new materials, and overall new paradigms for organizing and controlling complex systems.

That is why bioengineering — along with data science, another foundational tool with vast potential — has become a deep strength at Princeton and is one of our highest priorities for growth going forward. Faculty and students across the engineering school are pushing the boundaries of bioengineering and health in close collaborations with biologists, chemists, neuroscientists, data scientists, physicists, and clinicians.

Here is how one of our leading bioengineers and the recipient of a MacArthur "Genius Grant," Cliff Brangwynne, put it recently:

"What evolution has given us in biological systems are the most complex forms in the universe. So by studying biological systems in general, or unbelievably organized ones like the brain in particular, you can learn a lot about what is possible in terms of engineering and controlling systems with totally new kinds of properties."

In this magazine we look at just a slice of bioengineering research at Princeton, with a particular focus on work at the level of cells and cell systems, which is one of our core areas of excellence.

Are you working in bioengineering or health, or fields affected by them? Follow us on social media and tell us the stories of your work.

H. Vincent Poor *77

Interim Dean

Michael Henry Strater University
Professor of Electrical Engineering

Photo of H. Vincent Poor
by David Kelly Crow

Cover: Watercolor rendering of
lung tissue from developing
mice as studied in the lab of
Celeste Nelson (see page 8).
Art by Matilda Luk.

Contents

1
News

8
**Bio
Plus**



PROTECTING SMART MACHINES FROM SMART ATTACKS

The ability of machines to learn by processing sensor data — which underlies automated vehicles, medical devices, and a host of other emerging technologies — leaves systems vulnerable to hackers in unexpected ways.

A Princeton team has explored how adversarial tactics applied to artificial intelligence (AI) could, for instance, trick a traffic-efficiency system into causing gridlock or manipulate a health application to reveal patients' medical histories.

“For machine learning technologies to achieve their full potential, we have to understand how machine learning works in the presence of adversaries. That’s where we have a grand challenge,” said Prateek Mittal, the lead researcher and an associate professor of electrical engineering.

One type of threat, called an evasion attack, assumes a machine learning model has successfully trained on genuine data and achieved high accuracy at its task. An adversary could turn that success on its head, though, by manipulating the inputs the system receives once it starts applying its learning to real-world decisions.

For example, the AI for self-driving cars is trained to recognize traffic signs, while ignoring signs such as fast food restaurant logos. Mittal’s group has explored a loophole whereby signs can be misclassified if they are marked in ways that a human might not notice. The

researchers made fake restaurant signs with extra color akin to graffiti or paintball splotches. The changes fooled the car’s AI into mistaking the restaurant signs for stop signs. In other examples, they altered stop signs to be perceived as a variety of other traffic instructions.

The researchers have also looked at the threat of privacy attacks, which try to piggyback on machine learning models as the models soak up data, gaining access to guarded information such as credit card numbers, health records, and users’ physical locations.

An example of this malfeasance is the “membership inference attack.” It works by gauging whether a particular data point falls within a target’s machine learning training set. For instance, should an adversary alight upon a user’s data while picking through a health-related AI application’s training set, that information would strongly suggest the user was once a patient at the hospital. Connecting the dots on a number of such points could disclose identifying details about users and their lives.

“We’re entering a new era where machine learning will become increasingly embedded into nearly everything we do,” said Mittal. “It’s imperative that we recognize threats and develop countermeasures against them.”

– by **Adam Hadhazy**

Arjun Nitin Bhagoji and Liwei Song, graduate students in electrical engineering, are developing defenses to protect artificial intelligence from hackers. This stop sign has been modified to trick systems relying on computer vision. (Photo by David Kelly Crow)

CONTROLLING METHANE IS A FAST AND CRITICAL WAY TO SLOW GLOBAL WARMING

In independent studies, two Princeton University research teams identified surprisingly large sources of methane, a powerful greenhouse gas, being leaked into the atmosphere. Pound for pound, methane causes a far greater warming effect in the atmosphere than does carbon dioxide — 86-fold more heating over 20 years, and 35-fold more over the course of a century.

In one study, in the journal *Environmental Science and Technology*, a team headed by Mark Zondlo, associate professor of civil and environmental engineering, looked at an area around western Pennsylvania rich with natural gas wells and found that a small number of these wells are “superemitters” of methane. The other study, published in *Atmospheric Chemistry and Physics*, came from the re-

search group of Denise Mauzerall, a professor jointly appointed in civil and environmental engineering and the Woodrow Wilson School of Public and International Affairs. By equipping fishing boats with sensors and sailing around offshore oil and gas rigs in the North Sea, the researchers found that these facilities leak substantially more methane than previously reported.

Just after these studies were released, the Trump administration announced plans to roll back restrictions on methane emissions.

A question-and-answer story with Mauzerall's and Zondlo's views on methane and regulation can be found at <https://engineering.princeton.edu/controlling-methane>. – **by Steven Schultz**

Researchers in Mark Zondlo's lab created a mobile laboratory, the Princeton Atmospheric Chemistry Experiment, to measure the concentration of gases in the air near natural gas wells. Dana Caulton, a former postdoctoral researcher in the lab, is shown here in the mobile laboratory doing field sampling in the Marcellus Shale basin. (Photo courtesy of the Atmospheric Chemistry and Composition Group)





PRINCETON RESEARCHERS WORK TO LESSEN GLOBAL NUCLEAR DANGER

Researchers with Princeton University's Program on Science and Global Security have received multi-year grants from the American Physical Society and the Carnegie Corporation of New York to support work toward reducing nuclear risks. The grants will support technical and policy analysis to reduce the risks from nuclear weapons and material, respond to the rapid erosion of arms control, and assess the impact of emerging weapons technology. The support also aids efforts to re-engage the U.S. physics community on the need to reduce the threat of nuclear weapons.

The impulse for the effort is the worsening global nuclear threat, according to the Princeton group. The world has roughly 10,000 operational nuclear warheads, mostly held by the United States and Russia. It includes about 2,000 warheads on alert status, capable of launch within minutes. There are numerous new dangers, such as U.S. withdrawal from several important arms control treaties, a buildup of offensive capabilities by Russia and China in response to the growth of U.S. ballistic-missile defense, and cyberthreats to nuclear command and control systems.

The American Physical Society's grant supports efforts by a group led by members of the Program on Science and Global Security to interact with the U.S. physics community through visits to physics institutions including universities, national labs, industry, and conferences. To benefit from a diverse set of perspectives and talents, the project will establish fellowships for early-career physicists, with a particular focus on diversity and inclusion of women and underrepresented minorities.

The Carnegie award targets three initiatives. It will support the International Panel on Fissile Materials, an expert group founded in 2006 and currently led by Alexander Glaser, an associate professor of mechanical



and aerospace engineering and international affairs, and Zia Mian, a Princeton research scientist, who are co-directors of the Program on Science and Global Security. The panel works through international organizations to verifiably end production of plutonium and highly enriched uranium. The grant will also support work to define an adaptive architecture of nuclear weapons restraint and security to reduce the risk of strategic conflict. Finally, the grant will help assess the potential impacts of emerging technology on global security.

Founded in 1974, the Program on Science and Global Security conducts scientific, technical, and policy research to advance policies for a safer and more peaceful world. Focus areas include nuclear technology, biosecurity, information and communications technologies, artificial intelligence, autonomous weapons, and space-based systems. – **by B. Rose Huber**

Video still of a simulation developed to depict nuclear war between the United States and Russia using realistic nuclear force postures, targets, and fatality estimates. It is estimated that there would be more than 90 million people dead or injured within the first few hours of the conflict. Video available at <https://www.youtube.com/embed/2jy3JU-ORpo>. (Image courtesy of the Program on Science and Global Security)



REAL-LIFE EXAMPLES BRING NEW ENERGY TO CORE THERMODYNAMICS COURSE

Lamyaa El-Gabry (second from left), a lecturer in mechanical and aerospace engineering, teaches the department's introductory thermodynamics course. This fall, students toured the Public Service Enterprise Group (PSEG) generating station in Sewaren, New Jersey, with PSEG engineer Christian Santoro (far left). (Photo by Tori Repp/Fotobuddy)

Traditionally, engineering students have learned about the thermodynamics of gas turbines by studying diagrams and solving equations, but last fall they also donned hard hats, safety glasses, and ear plugs to tour a plant that produces electricity for half a million homes.

Long seen as a rite of passage for sophomores majoring in mechanical and aerospace engineering, the thermodynamics course is known for its rigor. In 2019, the class became a Campus as Lab course, featuring field trips, guest lectures, and lessons that enhanced students' learning with examples of energy technology and policy.

Besides visiting the Public Service Enterprise Group (PSEG) generating station in Sewaren, New Jersey, about 30 miles from Princeton, students joined professional engineers to assess the energy efficiency of the University's Pink House, home to undergraduates focused on sustainable living. Guest lecturers included an engineer who helps design gas turbines for extreme conditions and a contributor to the town of Princeton's Climate Action Plan.

"In the past, I would present all the theory and then talk about applications at the end," said course instructor Lamyaa El-Gabry, a lecturer in mechanical and aerospace engineering. But she worried that students were "sitting there getting heaps of equations," waiting to learn about "the cool stuff."

El-Gabry revamped the course with a focus on local dimensions of global energy issues, with help from campus sustainability manager Caroline Savage (now program director for Princeton Internships in Civic Service at the Pace Center for Civic Engagement) and Leah Anderson, associate director of the Program for Community-Engaged Scholarship.

The course's new emphasis inspired assignments that helped students connect concepts of thermodynamics to everyday life — such as campus energy plant director Ted Borer's explanation of the chilled water system that provides air conditioning to campus buildings. Many students used independent projects to pursue questions of energy efficiency. One group built a heat exchanger to test a method of increasing efficiency by capturing heat that would otherwise be wasted — for example, the exhaust from a clothes dryer. Another designed a device to measure the heat-insulating properties of different wood varieties. In their experiments, cedar wood allowed less heat to escape than fir or pressure-treated pine, which is commonly used in building frames.

Thomas Van Liere, a sophomore who worked on the second project, said he appreciated that the course was "more than just math. We learned about practical applications in industry, and jobs where we might use this knowledge." — **by Molly Sharlach**



GLOBAL WINDS REVERSE DECADES OF SLOWING AND PICK UP SPEED

In a boon to wind farms, average daily wind speeds are picking up across much of the globe after about 30 years of gradual slowing. Princeton-led research shows that wind speeds in northern mid-latitude regions have increased by roughly 7% since 2010.

The findings mark a reversal of the pattern of declining winds in these regions since the 1980s — a phenomenon known as global terrestrial stalling. Focusing on regions of North America, Europe, and Asia where wind power is on the rise, the researchers analyzed wind speed records collected between 1978 and 2017 from more than 1,400 weather stations. In a paper published in *Nature Climate Change*, they showed that while wind speeds decreased by about 2.3% per decade beginning in 1978, since 2010 wind speeds have

increased at a rate that is nearly three times faster. Zhenzhong Zeng led the study as a postdoctoral researcher working with Eric Wood, Princeton's Susan Dod Brown Professor of Civil and Environmental Engineering, Emeritus.

Zeng and his colleagues used statistical methods to test associations between variations in wind speed and an array of well-characterized ocean-atmosphere oscillations. The analysis showed that in each region, specific large-scale ocean-atmosphere oscillations, which are driven by many factors including the uneven heating of the earth's surface in different regions, were likely explanations for the observed trends in wind speeds. — **by Molly Sharlach**

NEWS



Wind turbines rise above Ningbo on China's Pacific coast. (Photo by Erping Sun)

MICROBE CHEWS THROUGH PFAS AND OTHER TOUGH CONTAMINANTS

In a series of lab tests, a relatively common soil bacterium has demonstrated its ability to break down the difficult-to-remove class of pollutants called PFAS.

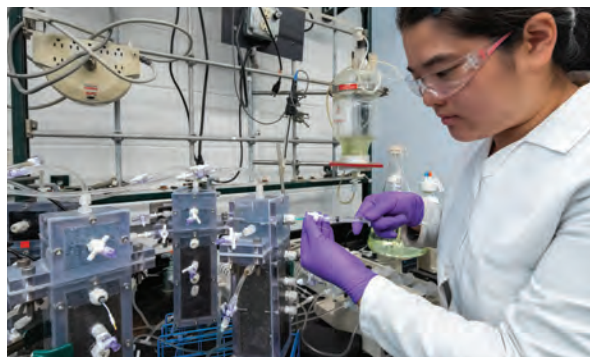
The bacterium, *Acidimicrobium* A6, removed 60% of PFAS — specifically perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) — in lab vials over 100 days of observation, the researchers reported in an article in the journal *Environmental Science and Technology*. Because of their health concerns and ubiquity, the Environmental Protection Agency has recently opened a research effort

into the chemicals' impact on drinking water. Peter Jaffé, the lead researcher and the William L. Knapp '47 Professor of Civil Engineering, said the researchers were very encouraged to see these bacteria substantially degrade the famously recalcitrant class of chemicals, but cautioned that more work was needed before reaching a practical treatment.

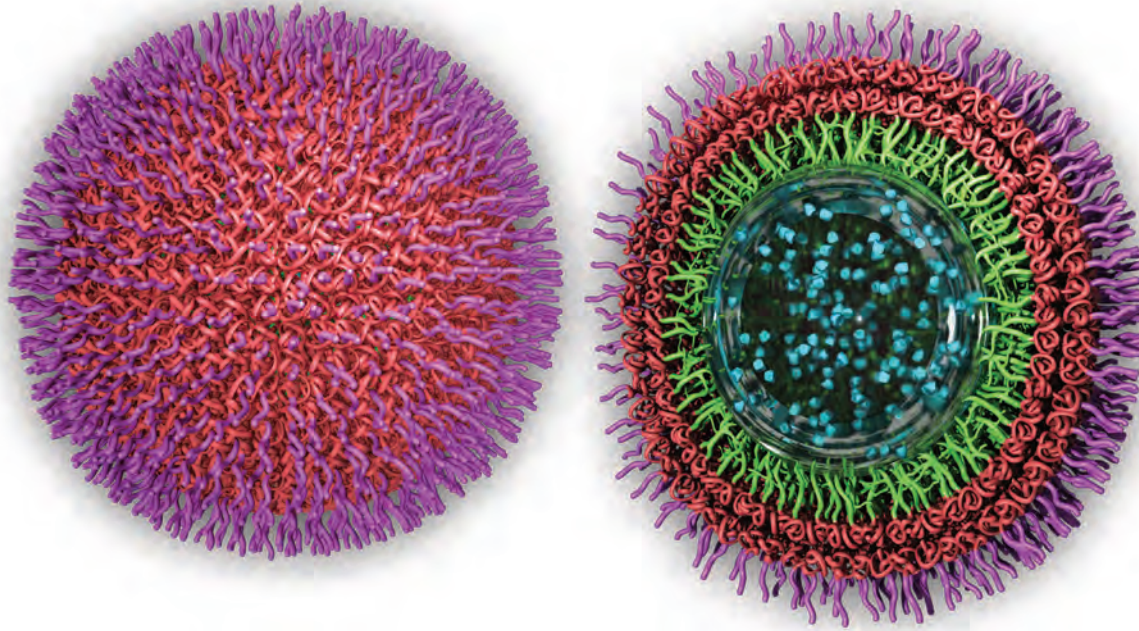
"This is a proof of concept," Jaffé said. "We would like to get the removal higher, and then go and test it in the field." — **by John Sullivan**

Left: *Acidimicrobium* A6 was able to remove 60% of a difficult-to-remediate class of contaminants called PFAS. (Photos by David Kelly Crow)

Right: Shan Huang, a professional specialist at Princeton, works with a reactor used to evaluate the bacterium.



IT'S ALL IN THE DELIVERY — NANOPARTICLE PLATFORM COULD TRANSFORM MEDICAL TREATMENTS



An artist's rendering of Optimeos' nanoparticle system. Developed in Robert Prud'homme's lab, the system is a vehicle for delivering medications to precise locations in the body or the interior of cells to treat myriad diseases, including cancer and diabetes. (Illustration by Rachel Davidowitz)

The startup Optimeos Life Sciences, founded by two Princeton faculty members, has reached agreements with six major pharmaceutical companies to develop therapeutics using a Princeton-developed drug delivery technology. The partnerships have the potential to improve the effectiveness of medications for the treatment of diseases ranging from cancer to diabetes.

Optimeos, founded in 2016 by Robert Prud'homme, a professor of chemical and biological engineering, and Shahram Hejazi, a faculty member in the Keller Center for Innovation in Engineering Education and in electrical engineering, focuses on bringing technology developed over 15 years in Prud'homme's lab to market. The technology, called flash nanoprecipitation, enables the encapsulation of drugs into nanoscale particles that improve delivery and effectiveness.

The new venture extends the impact of the technology, which already is being used in a project with the Bill and Melinda Gates

Foundation. The foundation awarded Prud'homme's lab a \$1.2 million grant in 2016 to apply their technology to increase the effectiveness of drugs used in global health. Solutions for global health problems have to be low-cost and robust, and the flash nanoprecipitation process allows for the creation of medications that are both. The method has been applied to three drugs sponsored by the Gates Foundation: a drug to treat diarrhea in infants caused by drinking polluted water, a tuberculosis drug, and a single-dose treatment for malaria.

Optimeos' new agreements involve creating improved delivery methods for six different medicines. The names of the six biopharmaceutical companies are currently undisclosed due to the proprietary nature of the ventures. The targets for the various projects are immuno-oncology, autoimmune diseases, diabetes, diseases of the central nervous system, and ocular diseases.

The drug companies now working with Optimeos need methods for delivering highly soluble biologics — a class of structurally complex therapeutics including proteins, peptides, and nucleic acids. Biologics have higher potency and fewer side effects than other treatments, but their physical characteristics require delivery through frequent injections. Biologics are also limited in what ailments they can treat unless the medicines are manufactured with sophisticated formulations.



“The future of therapeutics are potent biological drugs, many of which have delivery challenges with respect to how much drug needs to be delivered to exactly where in the body, while minimizing side effects,” Hejazi said.

Optimeos offers a solution to both problems. Using the flash nanoprecipitation method, in which streams of materials combine in specially engineered mixing chambers, the company is able to create medicines that slowly release therapeutic material inside the body over a period of weeks or months. This reduces or eliminates the need for repeat injections. The method also substitutes for the complex formulations required for other medications but at much greater speed and lower cost.

One application of these slow-releasing particles is in the treatment and management of diabetes. In 2019, Optimeos received funding through a National Science Foundation grant to develop a once-monthly injection of liraglutide, a non-insulin drug used in the treatment of type II diabetes and obesity. Liraglutide is currently administered by a daily injection. The new formulation under development by Optimeos aims to reduce the total amount of drug needed, reduce side effects, and reduce the frequency of injections. These attributes enhance patient comfort, adherence, quality of life, cost of care, as well as medical outcomes, said Robert Pagels, director of research and development for the company and a 2018 Ph.D. graduate from Prud’homme’s lab.

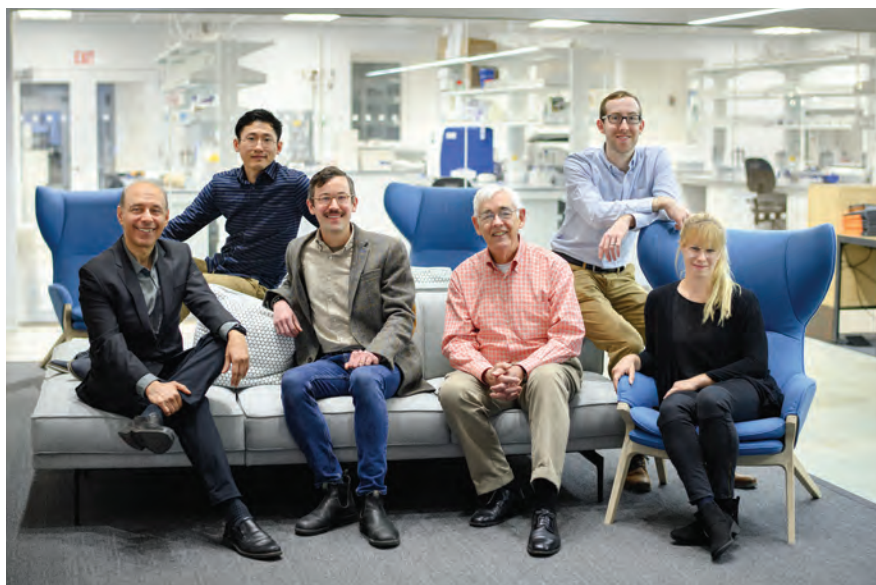
The flash nanoprecipitation method is able to achieve these results because of the way it encapsulates biologics into nanoparticles and microparticles. First, in their lab, the scientists make primary nanoparticles in which the drug-filled core is covered by a skin of specially designed polymers. Then these primary nanoparticles can be coated with additional polymers that are engineered to interact with

specific tissues or cells in the body. Finally these coated nanoparticles are applied to targeted drug delivery and intracellular delivery of biologics.

For some uses, the primary nanoparticles can be further assembled into larger composites, much like a cluster of grapes. These composites will slowly release the encapsulated therapeutic over a period of weeks to months.

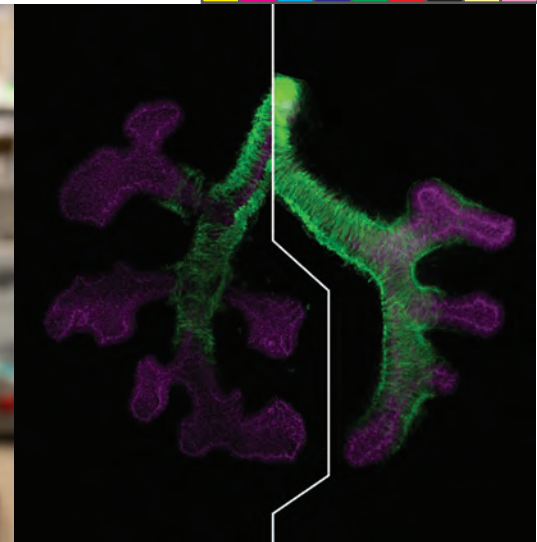
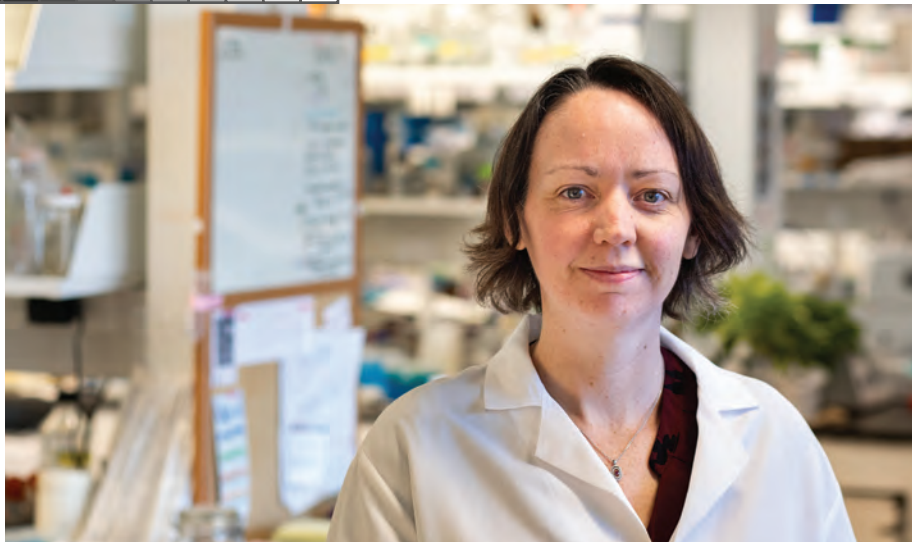
Prud’homme sees Optimeos bringing his scientific goals to fruition. “My academic research has focused on understanding the fundamental principles behind polymer assembly and processing to enable us to make elegant nanoparticles,” he said. “However, as an engineer, I also want to do something that can make an impact on human health, as opposed to just trying to do a one-shot thing that is beautiful and advances science.”

– by **Amelia Herb**



A startup founded by two Princeton faculty members has agreements with pharmaceutical companies to bring a nanoscale drug delivery system to market. Pictured at Princeton Innovation Center BioLabs, team members from left: Shahram Hejazi, Bumjun Kim, Robert Pagels, Robert Prud’homme, Chester Markwalter, and Madeleine Armstrong. (Photo by Sameer A. Khan/Fotobuddy)





NATURE REVEALS THERE'S MORE THAN ONE WAY TO BUILD A LUNG

by Molly Sharlach

Above left: Photo of Celeste Nelson by Tori Repp/Fotobuddy. Right: Nelson's research on mammalian lung development has shown that smooth muscle cells shape the growth of the very earliest airway branches. Branching morphogenesis of the airway epithelial cells (magenta) is guided by patterned differentiation of airway smooth muscle (green). Experimentally enhancing smooth muscle differentiation restricts epithelial branching (right panel), while inhibiting smooth muscle differentiation leads the epithelial cells to dilate and buckle (left). (Image by Katie Goodwin)

Our bodies are home to hidden trees — complex, branching structures vital to the functions of organs including the lung, kidney, and pancreas.

Celeste Nelson explores how branching patterns emerge during development. Her research combines biology with engineering and computational modeling — with the ultimate goal of building functional tissues outside the body.

“These branched architectures are ubiquitous,” said Nelson, a professor of chemical and biological engineering. “In the lung, they’re important for making sure there’s enough surface area to move oxygen from a breath into the bloodstream,” while in organs like the mammary or salivary glands, “it’s kind of a space-filling mission: making enough milk, making enough saliva.”

Much of Nelson’s work is focused on the forces that cause a clump of cells in the embryo to grow into the tree-like pattern of airways in the newborn lung. To understand how different physical processes can achieve similar results, her group has investigated lung development not only in mice, a model for human development, but also in birds and reptiles.

Nelson’s lab has spent years uncovering the details of mouse lung development, finding that physical signals from smooth muscle cells direct cells lining the developing airway to split into two branches — a process that occurs millions of times to form each well-functioning newborn lung. And more

recent work led by graduate student Katie Goodwin has shown that smooth muscle cells also shape growth of the very earliest airway branches, which form when a bud begins to grow on the side of a main branch (versus the end of a branch splitting in two).

Reptile lungs, in contrast, are simpler sacs that lack elaborate branching patterns. In developing embryos, smooth muscle cells form a hexagonal mesh that wraps around the growing lung, contracting and squeezing the epithelial cells to form the folds of the mature organ. In a collaboration with Andrej Košmrlj, an assistant professor of mechanical and aerospace engineering, and Jared Toettcher, an assistant professor of molecular biology, Nelson has received support from Princeton’s Eric and Wendy Schmidt Transformative Technology Fund to develop a 3-D printing technology for artificial organs based on knowledge of reptile lung development.

“Can we take what we’ve learned from looking beyond conventional model systems — learning how various species build their lungs — to come up with new ways to engineer three-dimensional structures outside of the body?” Nelson asked. “We’ll see how far we can go with this idea of one contractile tissue like muscle pushing on another to force it to change shape.” **E**



Stanislav Shvartsman *99 dreams of modeling every miniscule interaction in the embryo in all its tortuous detail.

But building a complete, dynamic model of development requires total control over the chemical, physical, and biological processes that govern life's first unfolding.

"Development is reproducible and precise," said Shvartsman, a professor of chemical and biological engineering and the Lewis-Sigler Institute for Integrative Genomics. "It's a program. We try to understand the differential contributions of physical laws and genomic instructions."

To gain insight into how this program works, from the molecule to the organism, Shvartsman's lab combines genetic, biochemical, and imaging experiments with biophysical computer simulations. The team often devises clever ways to break developmental processes and measure the outcomes, teasing out cause and effect.

A recent study used light and specially sensitized zebrafish embryos to understand how proteins carry information from the outside to the inside of a cell. In a normally developing embryo, chemical signals travel along tightly regulated pathways into and out of the nucleus. By flooding the embryo with light, the researchers manipulated these pathways to be overactive, which allowed them to trace inputs and outputs over time. The links they discovered between proteins and tissue dynamics illuminated processes connected to, for example, congenital heart defects and learning disabilities.

Typical of the Shvartsman lab, this work turned a biological process around to reveal deep truths about how life forms and functions.

"I had the insight to use what's already there in nature — a property of a mutation in an enzyme — to engineer a new approach to study biology," said graduate student Aleena Patel, who led the study.

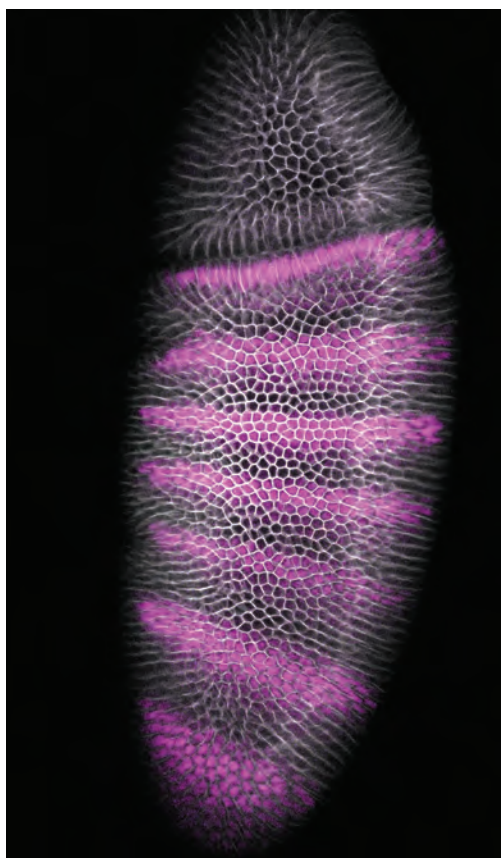
Another project, led by then-graduate student Jasmin Imran Alsous (now a postdoctoral researcher at MIT), looked at developing fruit fly eggs from a mechanical point of view —

a 3-D object packed with smaller 3-D objects. How does complexity emerge in those first few steps of life? The researchers turned to a branch of mathematics called graph theory and found that some packing configurations were more likely, and beneficial, than others. When the favorable configurations were disrupted, the embryos stopped developing — a finding that revealed profound connections between geometry and developmental dynamics.

Through 3.5 billion years of evolution, nature's program has become a marvel of efficiency. While Shvartsman would like nothing more than to decode it from start to finish, he also stands in awe of its power. "Development is magical," he said. "Whether fly or human." **E**

SOLVING THE PUZZLE OF LIFE'S DEVELOPMENT

by Scott Lyon



Proteins in a fruit fly embryo have been stained pink and photographed with a special microscope, enabling Stanislav Shvartsman's team to trace in minute detail how the expression of pair-rule genes informs cell behavior as the insect develops. (Image courtesy of the researchers)

INTERPRETING THE GENOME FOR INSIGHTS INTO LIFE AND HEALTH

Olga Troyanskaya uses computing power to glean useful insights from an enduring mystery: How can the genome, the vast set of instructions that lies within every cell, be interpreted to understand, prevent, and treat disease?

Since the human genome was decoded in 2003, scientists have labored to relate its 3 billion bits of information to health and illness. Using machine learning and artificial intelligence, the Troyanskaya lab sheds light on the entire genome and the networks of interactions within it. Their work has produced important insights into cancer, autism, heart disease, and other disorders.

In one recent study, the group analyzed the genomes of nearly 1,800 families and found thousands of mutations that could contribute to autism. These mutations were not in genes themselves, but in the largely uncharted stretches of DNA that regulate how genes function.

In another area of work, the lab has analyzed more than 10,000 publicly available data sets and created hundreds of maps of cells and circuits that allow scientists to predict how the effects of mutations would ripple through the many processes in various tissues or organs, such as the brain or liver.

“Data-driven machine learning approaches are absolutely critical for turning the vastness of publicly available data into knowledge for health and medicine,” said Troyanskaya, a professor of computer science who is jointly appointed at Princeton’s Lewis-Sigler Institute for Integrative Genomics and the Simons Foundation in New York. – **by Steven Schultz**



Professor Olga Troyanskaya (left) speaks with Chandra Theesfeld, an experimental biologist in her lab, which combines computer science and biology to better interpret the human genome and its role in disease. (Photo by Sameer A. Khan/Fotobuddy)

THWARTING BACTERIA’S DEFENSES AS A WAY TO FIGHT INFECTIONS

To combat the growth of antibiotic resistance, Mark Brynildsen searches for precise treatments that kill pathogens by weakening their defenses against the body’s immune system.

“We study how bacteria defend themselves against the stresses imposed by the immune system, in order to identify ways to sabotage some of those defenses,” said Brynildsen, an associate professor of chemical and biological engineering.

For instance, some immune cells deploy nitric oxide, a highly reactive molecule that helps kill bacteria by damaging their proteins and DNA. But many bacteria, including food-borne pathogens such as *E. coli* and salmonella, make enzymes that can detoxify nitric oxide before it causes much harm. In addition to probing the genetic mechanisms that allow bacteria to survive nitric oxide, Brynildsen’s lab is working with Princeton’s Small Molecule Screening Center to search for compounds that might interfere with the bacteria’s defenses.

“This defense system is important for a pathogen to cause an infection, so that suggests that if you can drug that defense system you can actually get a treatment that’s not an antibiotic,” in the sense that it does not directly kill bacteria or stop their growth, said Brynildsen, whose work in this area is funded in part by Princeton’s Helen Shipley Hunt Fund. “It works in conjunction with your immune system.” And because of its specificity, “if it gets out of your body it’s not going to cause drug resistance development in water or sewage or agricultural settings.”

– **by Molly Sharlach**





To combat the growth of antibiotic resistance, researchers in Mark Brynildsen's lab are searching for precise treatments that kill bacterial pathogens by weakening their defenses against the body's immune system.
(Photo by David Kelly Crow)

'EVOLUTION ACCELERATORS' SHOW HOW SOME CANCERS EVADE TREATMENT

James Sturm '79 creates devices with microscopically small structures, forging new tools to manipulate living cells and control their environments.

Working with biophysicists and medical researchers, Sturm's group has developed microchips that allow them to closely follow the progression of cancer and investigate drug resistance, a major problem in cancer treatment.

"We're making little Galapagos Islands-type environments on a chip," said Sturm, Princeton's Stephen R. Forrest Professor in Electrical Engineering. Each chip contains 100 interconnected "microhabitats" — tiny chambers, each with slightly different drug concentrations — and cells can move freely between them, like birds flying from one island to another.

This mimics the varied environment of a tumor, where drug concentrations depend on proximity to the bloodstream and other factors. "We have found that this heterogeneity is fundamentally important in how drug resistance evolves," said Sturm.

Sturm's group collaborated with Professor of Physics Robert Austin and researchers from the Johns Hopkins University School of Medicine to develop the microfluidic devices, which they call "evolution accelerators."

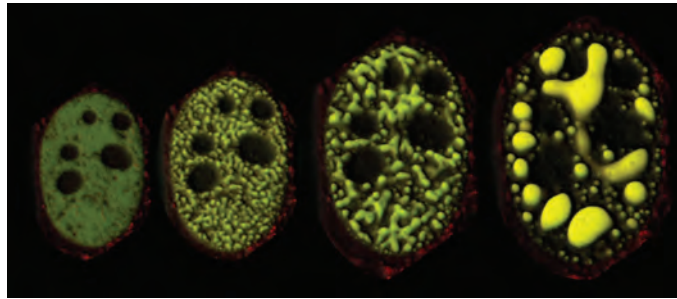
Among other projects, the researchers have used the technology to examine prostate cancer cells and explore factors that allow certain cancerous cells to survive chemotherapy treatments. In one example, they have found that giant cancer cells, which contain multiple copies of the genome, arise quickly only where drug concentrations are locally high but lower nearby. Further, these cells have properties that may contribute to spread of cancer. By observing this transition in real time, researchers hope to identify new ways to "break the link" between drug resistance and metastasis, said Sturm. — **by Molly Sharlach**



James Sturm '79 (Photo by David Kelly Crow)

DROPLETS ARE KEY TO CELLS' DYNAMIC ACTIVITIES

by Molly Sharlach



Clifford Brangwynne's team has developed novel tools to examine and manipulate how proteins condense into specialized droplets, and is applying these approaches to uncover new aspects of gene regulation. (Image courtesy of the researchers)

A cell is a complex machine whose flexible architecture enables it to take part in life's activities: relaying information, managing waste, responding to stress.

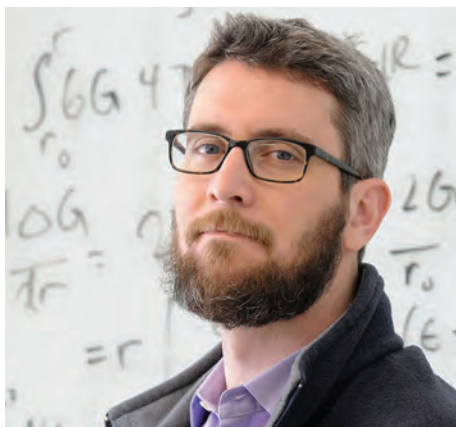
One way that proteins and other molecules in the cell achieve this flexibility is by condensing into specialized droplets, a phenomenon called liquid-liquid phase separation. Clifford Brangwynne, a professor of chemical and biological engineering, leads a research group investigating the formation and functions of these droplets — called membraneless organelles or condensates, in contrast to the better-known membrane-enclosed organelles such as the mitochondria or nucleus.

Membraneless organelles play key roles in the nucleus, where a cell's genome is stored and its genes are transcribed into messages that direct cellular activities. Brangwynne's team has developed novel tools to examine and manipulate the condensation of proteins in the nucleus, and is now applying these approaches to uncover new aspects of gene regulation. The work could help elucidate how gene regulation goes awry in cancer and neurodegenerative diseases, and may aid in the discovery of new therapeutic approaches.

"The genome is not just an abstract software program," said Brangwynne, a Howard Hughes Medical Institute Investigator. Each chromosome is a long strand of DNA that's packed into a relatively tiny nucleus and wrapped around accompanying proteins. Physical forces are critical to gene regulation, and Brangwynne explores how protein droplets act in, on, and around the genome to influence the expression of genes.

To this end, Brangwynne and colleagues created a technique called CasDrop. It combines optogenetics, or the use of light to control cellular activities, with CRISPR gene-editing technology to activate the condensation of proteins at specific DNA sequences within a cell's genome. CasDrop allowed the researchers to begin studying the biophysics of the droplets' interactions with the genome, revealing how protein droplets are attracted to open, active regions of the genome, and how droplets can pull distant parts of the genome together to activate genes in a coordinated fashion.

"These droplets are deforming the genome, and the way in which that's orchestrated to turn on some genes and turn off others is a very active area of research," said Brangwynne. "You have many different types of droplets and they can be immiscible with one another — in other words, not able to mix — and you can generate all kinds of interesting structures. We try to think about organization of the genome and expression of the genome and how physical forces associated with these droplets are restructuring it." **E**



Clifford Brangwynne (Photo by Laura Pedrick for HHMI)



For José Avalos' research team, the intricate, small-scale work of bioengineering could hold the key to solving global challenges in renewable energy and sustainable manufacturing.

"Our minds are in biotechnology, but our hearts are in helping to improve the health of the planet," said Avalos, an assistant professor of chemical and biological engineering and the Andlinger Center for Energy and the Environment.

Along with his postdocs and students, Avalos wields an array of tools, including light-activated genes, to tailor the metabolisms of yeast and bacteria. These modified microbes go on to produce chemicals of interest such as fuels, commodity chemicals, and drug precursors. The overriding goal is to reduce, and eventually eliminate, our dependence on petroleum, the traditional feedstock for these materials.

"We want to move toward the concept of a 'bioeconomy,'" said Avalos, "where polluting fossil fuels are replaced by carbon-neutral biological processes."

More energy-dense and convenient for vehicle use than ethanol, the standard biofuel of today, isobutanol is unfortunately more toxic to yeast, which limits its production. In a study published in the journal *Cell Systems*, Avalos and his team revealed a fundamental mechanism of isobutanol's toxic effect. They found that in the presence of isobutanol, yeast behave like they are starving, even though they are in rich nutritious broth, causing them to slow their growth and metabolism. The team found a key gene that orchestrates this starvation response. Simply deleting this gene enabled the yeast to stave off the chemical's poisonous effects and pump out isobutanol at much higher levels.

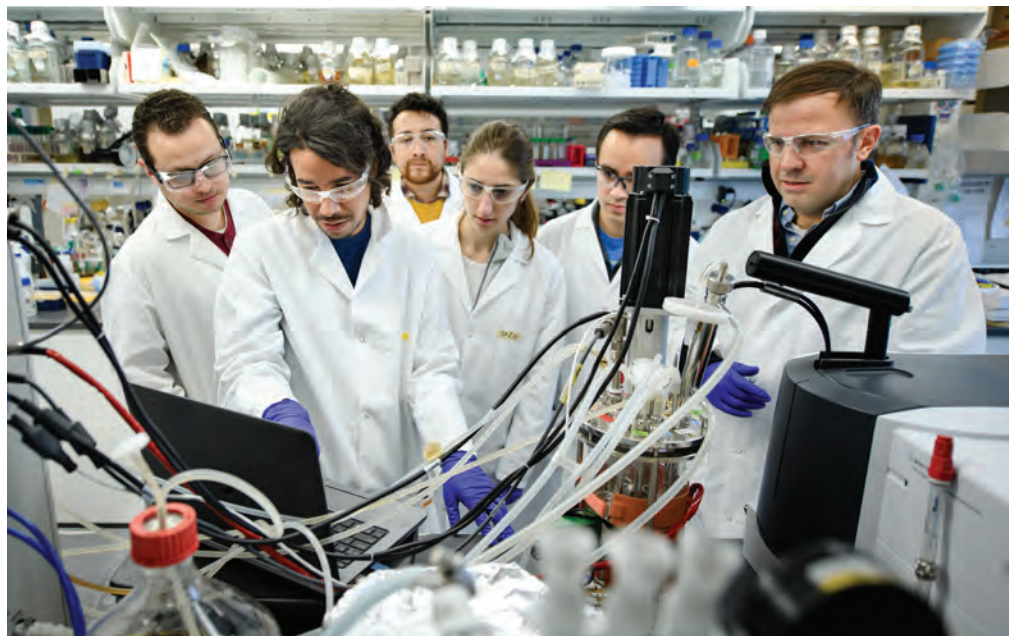
Delving into the nitty-gritty of microbial cells, from organelles to enzymes and metabolisms, has been the hallmark of Avalos' lab.

Researchers in his group engineer microbes, primarily yeast, to sustainably produce fuels and chemicals. The team works to fine-tune control of the microorganisms to turn on and off genes and to target the enzymes encoded by those genes to special compartments in the cell.

"Our goal is to make the cells more productive and to make renewable fuels more competitive with fossil fuels," Avalos said. "We are trying to drive the costs down, and the way we do that in the cells is by controlling when and where these genes are expressed." **E**

HARNESSING CELLULAR POWER TO MEET GLOBAL CHALLENGES

by Adam Hadhazy



José Avalos (right) and his research team engineer microbes to address global challenges in renewable energy and sustainable manufacturing. (Photo by Sameer A. Khan/Fotobuddy)



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