BIOTECHNOLOGY

David Liu's evolution workshop

Inside the lab that combines chemistry and evolution to try to cure disease

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David R. Liu is an inventor on a winning streak. As a young chemist, he quickly mastered techniques for studying life's most complex molecules and then turned to altering the building blocks of life itself. He repurposed the genetic code of DNA to create large libraries of small molecules for drug screening and created a system for engineering new proteins that's at least 100 times as fast as previous methods.

Most recently, Liu is leading the charge in conceiving improved versions of CRISPR, the gene-editing tool that is revolutionizing research. His most notable advance is a new "base editor" that can swap a single nucleotide base, or letter, of DNA for another. The invention opens the door to developing treatments for the thousands of genetic diseases caused by one small typo in DNA.

"He's going to be the godfather of CRISPR 2.0," says Gerald Joyce, director of the Genomics Institute of the Novartis Research Foundation.

What unites all this work in Liu's curious mind is a synthesis of chemistry and biological evolution. Liu isn't afraid to manipulate the structures of complex molecules, but is "constantly humbled" by how complicated it is to predict the outcome of these changes. "So that's why we acquiesce to the power of evolution to help guide our molecular discoveries," Liu explains. The goal is to combine a chemist's ingenuity with the Darwinian brutality of natural selection to access molecules that are more perfect than what man or nature alone could create.

Harvard University chemist Stuart Schreiber recalls a day in 2003 when Liu, a young professor there, began talking about drones. "Frankly, I didn't know what a drone was back then," Schreiber says. Liu showed him a small aircraft he built and began leisurely flying it around Schreiber's apartment. "He is one of the most intellectually curious persons I know," Schreiber says.

Joyce recalls a similar experience while visiting Liu's office many years ago. "It was sensory overload, with all the gadgets in his office and all the gadgets turning in his mind," says Joyce, who now works alongside Liu in Jason, an elite U.S. government scientific advisory group.

Liu's current office at the Broad Institute of MIT & Harvard is tamer. A gemstone collection, wooden bowls made with his lathe, and scenic photography have taken the place of whirring motors. But the engines in his mind are turning faster than ever.

At just 44, Liu has already spent nearly two decades directing young scientists at Harvard in high-risk, high-reward endeavors. In the past six months alone, Liu's group has landed four publications related to CRISPR in the pages of *Science* and *Nature*. "He has had blockbuster after blockbuster; it's just wild," Joyce says.

And Liu's discoveries aren't just academic curiosities. He has founded five companies, each based on a potentially transformative technology, a breadth of innovation that is typically reserved for engineers. They are calculated risks: Two of his ventures have failed, but the others seem poised to succeed.

In 2013, he became a scientific cofounder of Editas Medicine, a company developing CRISPR therapies for genetic diseases. He cofounded Pairwise Plants, a start-up unveiled last month with backing from Monsanto, to use his base-editing technology to engineer fruits and vegetables. Liu's fifth start-up, still in stealth mode, is set to launch soon. And he's working with a former graduate student on starting a sixth.

Liu is on track to become an important name in the biotech industry—a serial entrepreneur along the lines of a Robert Langer or Greg Verdine. He is reluctant to see himself in those terms, but everyone around him seems to recognize the potential lurking inside that insatiably thirsty mind.

Doug Cole, a managing partner at the venture capital firm Flagship Pioneering, remembers meeting Liu, then a new professor. "We walked out of his lab to get lunch, and we hadn't even gotten halfway across Harvard Yard before I was convinced that he was very likely to succeed," Cole says.

Calculated risks

Liu grew up in Riverside, Calif., his mother a physics professor and his father an aerospace engineer. "My parents never pushed me to be a scientist," Liu says, but his curiosity pulled him there anyway. In 1990, he won the California Junior Science & Humanities Symposium.

As a perk of winning, Liu got to attend the 1990 Nobel awards ceremony while a first-semester freshman at Harvard. In Stockholm, Liu met E. J. Corey, who had just won a Nobel Prize for his work in retrosynthetic analysis—the process of working backward to synthesize complex molecules found in nature.

The next semester, Liu began a research project in Corey's lab to unravel the details of an enzyme involved in cholesterol synthesis.

Liu's diligence in the lab was legendary. Once, after a full-day experiment failed, he worked all night to restart the project and hand it off to a grad student the next morning. "I was horrified," Corey recalls. But "it tells you something about David's fearless work ethic."

Liu not only graduated first in Harvard's class of 1994 but also submitted a synthesis of his undergraduate research "equivalent to a Ph.D. thesis," Corey says. "It was word perfect when he handed it in; there were no corrections needed." Corey's parting advice to the young scientist was to "not spend more time in grad school than he needed."

Liu traveled to the University of California, Berkeley, to study under Peter Schultz. Corey's lab "filled me with this amazing reverence for and belief in the power of synthetic chemistry," Liu says. But after training under a master in the art of recreating nature, he

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was eager to use chemistry to improve it.

At Berkeley, Liu forged methods for incorporating unnatural amino acids into the structures of proteins. Before Liu finished his Ph.D., Corey invited him back to Harvard to speak about his efforts to construct proteins with new properties.

Corey's colleagues were impressed. In 1999, fresh out of graduate school, Liu joined Harvard as an assistant professor. He was just 26.

With his own lab, Liu began exploring evolution on the molecular scale. His first big discovery as an independent scientist came out of a desire to appropriate the genetic code of DNA to allow scientists to create hundreds or thousands of new molecules that could be screened for drug activity. Liu quickly became a pioneer of DNA-templated synthesis, now commonly called DNA-encoded libraries, a method of appending small molecules to DNA strands to generate many combinations of new molecules.

Flagship's Cole worked with Liu to found a company called Ensemble Therapeutics in 2004 based on this work. The firm, which spurred the creation of several other companies using DNA-encoded libraries, closed last year.

One or two failures are expected from someone with a natural tendency to push boundaries—both professionally and personally. Early in his career, Liu was banned from MGM Grand casinos after a night in which he made too much money playing blackjack. Soon after, he began informally teaching a group of card-counting Harvard undergraduates. Over the course of four years, they developed nearly optimal solutions for winning the game.

"We went everywhere in the U.S.," Liu

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—Gerald Joyce, director, Genomics Institute of the Novartis Research Foundation

Liu's greatest hits

David Liu invents tools that combine the principles of chemistry and evolution. Here are some of his inventions that could have the greatest impact.

Expanded genetic code

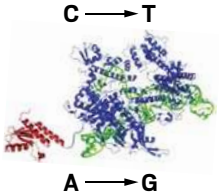
Early methods for incorporating unnatural amino acids into protein structures (*Proc. Natl. Acad. Sci. USA* 1999, DOI: 10.1073/pnas.96.9.4780)

DNA-templated organic synthesis

DNA sequences used to create a library of small-molecule macrocycles (*Science* 2004, DOI: 10.1126/science.1102629)

PACE

Phage-assisted continuous evolution to quickly engineer proteins with new properties (*Nature* 2011, DOI: 10.1038/nature09929)



Cytidine base editor

Modified CRISPR system that converts C:G base pair to T:A (*Nature* 2016, DOI: 10.1038/nature17946)

Adenine base editor

Modified CRISPR system that converts A:T base pair to G:C (*Nature* 2017, DOI: 10.1038/nature24644)

xCas9

PACE-evolved CRISPR enzyme capable of cutting at more DNA sites and at higher specificity (*Nature* 2018, DOI: 10.1038/nature26155)

says. "It was an amazing experience." He still keeps a case full of poker chips from their travels.

Work eventually curtailed his blackjack hobby, but Liu's propensity for taking risks continues in his lab, where he has created a space for ambitious students to pursue edgy—perhaps even ill-advised—ideas.

The lagoon

When Kevin Esvelt walked into Liu's office for the first time in 2004, the first-year graduate student made a bold request. Esvelt told Liu that he wanted a project "that no one else would be willing to try because it would be too ambitious."

Liu tasked Esvelt with something that was long on his scientific wish list: Develop a method for continuous protein evolution, a pressure chamber for accelerating the creation of synthetic proteins with traits that don't exist in nature.

At the time, scientists were already fashioning such proteins with directed evolution, a laborious technique that involved mutating large batches of genes, weeding out the protein progeny of interest, then repeating the cycle to arrive at a protein with the desired properties. A full cycle took about a week, meaning the kind of dramatic metamorphoses Liu envisioned could take years, even decades.

Esvelt proposed hijacking the 10-minute life cycle of bacteria-infecting viruses to run all the steps of protein evolution autonomously in a hot liquid chamber dubbed "the lagoon." In theory, the setup would allow the lab to quickly engineer proteins that could perform entirely new chemical reactions.

Esvelt couldn't get the idea to work in practice, though, and Liu began wondering how many years a responsible adviser should allow a student to continue with no successful results. "I was too naive as a young professor to know when to throw in the towel and try something else," Liu says.

After five and a half years, Esvelt got his system, named phage-assisted continuous evolution, or PACE, to work, allowing researchers to run dozens of rounds of evolution in a single day. Liu's lab has since used PACE to improve insecticidal proteins, evolve enzymes with new activity, and improve CRISPR gene editing.

"In retrospect I was a little bit delinquent," Liu says. Esvelt, however, loved the free rein. "David views it as his job to provide the space to let his students' creativity blossom," Esvelt says.

CRISPR 2.0

Liu's mentoring style has attracted a string of bright, young minds, eager to tackle all-or-nothing projects. "As Dave's career took off, it became harder for me to recruit grad students myself," says Schultz, Liu's Ph.D. adviser, now at Scripps Research Institute California.

When one prospective student told Schultz she would rather work for someone "younger and more dynamic," Schultz replied: "Well, chemistry is a little like learning the Force. Would you rather learn from Luke Skywalker or Yoda?" She went to Liu's lab.

Two other young researchers drawn to the Liu lab's ethos recently created what may be the group's most far-reaching discovery to date. When Alexis Komor was nearing the end of her Ph.D. with Jacqueline Barton at California Institute of Technology, she approached Liu about doing a postdoc in his lab to learn protein engineering. He encouraged her to brainstorm projects involving Cas9.

Cas9, the enzyme used in CRISPR gene editing, lines up with a specified sequence of DNA and cuts it, like a pair of molecular scissors. This allows scientists to make precise breaks in the genome, which is useful for research and certain therapeutic applications, such as turning a problematic gene off.

But Cas9 is not good at swapping DNA sequences. "I cringe when I read stories about how CRISPR allows us to change any DNA sequence into any other DNA sequence, which is not correct," Liu says.

Liu and Komor devised a plan to make a new version of CRISPR that works more like a pencil and its eraser, changing a single DNA nucleotide from one letter to another—bona fide editing.

The project combines a version of Cas9 that binds DNA but doesn't cut it, an enzyme called a cytidine deaminase, and a third protein that altogether can convert a cytosine-guanosine (C:G) base pair into a thymine-adenine (T:A) base pair.

When Komor's cytidine base editor was published in 2016, another postdoc in the lab, Nicole Gaudelli, had already begun planning a base editor that worked in the opposite direction, from A:T to G:C. That was possible, in theory, by replacing the cytidine deaminase with an analogous enzyme called adenine deaminase. Gaudelli tried, but existing enzymes worked only on RNA, not DNA. That difference may seem trivial, but it meant that Gaudelli would have to think bigger if she wanted to develop the first adenine base editor.

Gaudelli dropped everything else she was working on to start an ambitious experiment: Use Esvelt's PACE to evolve an enzyme that doesn't exist in nature.

That broke Liu's long-standing rule: "If

CREDIT: PROTEIN DATA BANK (PROTEIN); SHUTTERSTOCK (DOUBLE HELIX & SCISSORS); GREG EMMERICH (MICROPHAGE); *NATURE* (RIBBON STRUCTURE)

step one is evolve your starting material, then don't pick that project." Even if they could evolve the enzyme, there was no guarantee they could coax it into editing DNA in cells.

"I don't think anybody could honestly say that we had high confidence that the project would succeed," Liu says. "We just anticipated that if it did succeed it would be really useful to the field, and potentially to patients."

After two years, Gaudelli wrangled PACE into creating the new base editor. Liu submitted the paper to *Nature* on the first Thursday in October 2017. He received three long referee reports back the following Monday. The paper was online 16 days later—a turnaround time that Liu still can't believe. Since then, another group has already designed, conducted, and published experiments using his team's adenine base editor in plants.

Impact

Just as CRISPR has abruptly transformed science, these base editors have changed Liu's life in ways he never would have expected.

Sarah Gladstone remembers the first time she heard about Liu's research. It was October, and the pediatrician was listening to the radio show "Science Friday" while driving her eldest daughter to a doctor appointment in Kansas City, Mo. Liu was on the air discussing his lab's base editors and their potential to cure genetic diseases. "I turned to my daughter and said, 'They are talking about you,'" Gladstone says.

Gladstone's daughter, now 12, has Wolfram syndrome, a rare condition caused by single-nucleotide mutations in a gene that leads to childhood diabetes, optic nerve atrophy, and eventually blindness. Gladstone emailed Liu, explaining her daughter's condition and wondering if the base editors could help.

Since base editors debuted in 2016, Liu receives one or two letters from parents like Gladstone every day. "It is both exciting and heartbreaking," he says. Liu has to explain to families that many hurdles lie ahead for base editing in humans, such as devising a way to deliver the bulky system into cells inside the body. But he says these interactions energize him and his students.

"It starts to feel more like we are doing something wrong if we are not doing everything we can to transition these scientific developments into therapeutics," he says.

Liu's legacy is already apparent in his students' successes. Gaudelli now hopes to translate her lab work into medicine at a stealth biotech start-up. Komor recently became an assistant professor at UCSD. And Esvelt, now an assistant professor at MIT, has garnered international attention for his work on CRISPR gene drive, a system to potentially engineer wild populations of mosquitoes and mice to eliminate malaria and Lyme disease. Still others have gone on to biotech start-ups, pharma companies, and venture capital firms.

In the past few years, Liu's lab has evolved from an incubator of promising science into a hub of transformative discoveries. He attributes his success to a "combination of being curious about a lot of things and having the incredible good fortune of having the kinds of students that I've been lucky to get."

Tenacity, fearlessness, and a bit of luck in the lab help too. Esvelt and Liu certainly learned that with PACE. "Sometimes nature will give you something beautiful on a silver platter," Esvelt says. "But the fact that David has had that happen several times to him, throughout his career, may indicate that it's not just luck." ■