Think about it: life, in all its infinite and exquisite complexity, for the most part derives from a mere 20 amino acids that, strung together in myriad ways, make the proteins that make us go. Nature’s pretty great, right?

For Alex Deiters, who’s on the faculty at the University of Pittsburgh, great isn’t quite good enough.

Because he’s a chemist, he says, he finds “the functionality in those 20 amino acids very limited.”

And because he’s a chemist, he chooses to make new ones.
To understand Deiters’s motivations, it’s important to appreciate why anyone studies biological pathways and processes, from cell signaling to gene expression, in the first place: knowing at the molecular level how these processes work—and why they sometimes get tripped up—is essential to improving human health and understanding fundamental questions about life. (Like, Why did we all end up with 20 amino acids anyway?) One way to study these processes and the proteins involved is by manipulating them, then seeing what happens.

And scientists are getting pretty good at this. No doubt you’ve heard about that oddly named gene-editing technology, CRISPR-Cas9. The technology has been rocking the biomedical world for a few years—and it’s likely to rock our worlds, as well, before long.

If your genome is your body’s instruction manual and your DNA is the language it’s written in, CRISPR-Cas9 makes pretty neat editing software for fixing typos, adding new sentences, and deleting entire paragraphs. By pairing a Cas9 protein with a guide RNA, the system can be used to locate, remove, and replace specific DNA sequences.

You might want to do this with a mutant amino acid, thus effectively stopping Cas9 from snipping the targeted sequence.

Although Deiters finds Mother Nature lacking in imagination when it comes to amino acids, he admires her precision over where and when genes and proteins do their stuff. He’d like to offer scientists comparable sway by giving them the ability to place molecules under optical control.

Responsiveness to light is not intrinsic to proteins, so Deiters adds that function. To do this, he re-engineers the machinery of the cell to accept a light-responsive amino acid. This effectively expands the genetic code of a cell to 21 amino acids. The resulting extra and unnatural amino acid is modified with a light-removable chemical group that acts as a cage; the “cage” contains the amino acid so that it cannot perform its biological function. The caged amino acid is introduced to the protein at a selected site—in its catalytic center, say, or the area on the protein’s surface where it interacts with another protein. Once in place, the unnatural amino acid can then be exposed to UV light at a precise point in time. When that happens, the chemical photo-cage falls apart, and voilà: like a surprise guest popping out of a cake when the lights come on, the protein becomes active and starts doing what it is meant to do.

Deiters has already used this tool to place numerous biological processes under light control—he’s changed how proteins move around (translocate), influenced how cells signal, and coordinated vital molecular assembly lines like nucleic acid polymerization and DNA recombination.

Which brings us back to CRISPR-Cas9. Three years ago, Deiters brought optical control to bear on the system, inserting a photo-caged amino acid, thus effectively stopping Cas9 from snipping the targeted sequence.

And he recently published a paper with Bennett Van Houten, the Richard M. Cyert Professor of Molecular Oncology in the Department of Pharmacology and Chemical Biology, who studies DNA repair. By optically controlling a helicase, which unwinds DNA, they hope to provide insight into DNA repair pathways. That work could help answer questions related to cancer and aging.

Deiters considers CRISPR-Cas9 “a nice playground” for his photo-caging technology, but what really gets him excited is deploying his spatiotemporal superpower not in cell cultures, but in a living, breathing vertebrate, the zebra fish.

A few years ago, as Pitt was recruiting Deiters from North Carolina State University, he met Michael Tsang, an associate professor of developmental biology who studies cell signaling. Tsang is especially interested in how fibroblast growth factors (FGF) activate the pathways required for an embryo to grow. In humans, abnormal FGF signaling has been associated with cancers and congenital skeletal defects.

To explore this signaling, Tsang looks at zebra fish embryos. Researchers are fond of using the small, horizontally banded swimmers to study development and disease not only because they’re easier to house than mice, but also because they share 70 percent of our genes and have many of the same organs as we do. What’s more, the fast-growing embryo develops outside of the mother, and it’s transparent, providing a built-in window on the fish’s development—and on the impact of genetic manipulations.

“Alex always had an interest in bridging biology and chemistry, and I’m always game to try things out,” Tsang says, explaining how they came to join forces. The two began collaborating even before Deiters’s final arrival at Pitt in 2013.

In the next two years, they published on light-triggered nucleic acid molecules to con-
trol gene splicing and gene expression in zebra fish embryos.

In 2014, they received the Kaufman Foundation Initiative Award for their efforts.

By 2017, Deiters and Tsang had introduced a caged light-responsive unnatural amino acid into a zebra fish enzyme that controls what’s known as dorsoventral patterning.

“Dorsoventral patterning is a critical step in setting up the body plan of an embryo,” Tsang explains. “It is one of the first phases of development, where the embryo has to decide the front and back of the body.” If this patterning goes wrong, you may have, on one side, a central nervous system that’s too small and, on the other, a malformed gut.

By exerting optical control over the signaling that regulates this process, Deiters and Tsang were able to pinpoint the exact time window during which patterning is affected. The duo’s proof of concept, Tsang says, can (so to speak) shed more light on “the pathways that are involved in, say, heart development. If we ‘flip the switch’ [at a given moment], we can see if we can change that development.”

A converse approach is to create transgenic zebra fish lines with “mutations that replicate what happens in human disease, like cardiac defects,” says Tsang. “The question then becomes, If we flip the switch now, can we cure the disease? And, hopefully, that will show us when to activate these pathways [with drugs] to rescue that phenotype in humans.”

Introducing the brand-new amino acids into zebra fish has gone “remarkably well,” says Deiters. The fish “now have a genetic code that is expanded to 21 amino acids, where the 21st amino acid is synthesized by chemists and thus can be designed to have virtually any desired function—light activation being only one of them.”

Deiters says, “We’re improving the technology, making it more user friendly so others in the zebra fish community can adopt it.” He hopes to add more unnatural amino acids to the four he’s published about so far; he wants to control additional zebra fish proteins. And he wants to make the process reversible.

“Right now, we can turn [a protein] on but not off. It would be cool to generate a light switch that goes in both directions, at a given time point in a given location, and do it in multicellular model organisms.”
“Right now, we can turn [a protein] on but not off. It would be cool to generate a light switch that goes in both directions.”

Both Deiters and Tsang are quick to point out that what they make are investigative tools, but they acknowledge the possible exciting therapeutic applications for humans. Imagine being able to treat cystic fibrosis by delivering CRISPR-Cas9 to a patient, but activating it selectively, using a fiber-optic probe to irradiate only the lungs, for example.

Deiters also makes a point of distinguishing between this kind of genetic editing—treating distinct cell populations, such as tumors, the lining of the respiratory tract, or blood cells—from altering the germ line, which refers to copies of the genome that may be passed down through generations. In a position statement for the National Institutes of Health, director Francis Collins calls the latter “a line that should not be crossed,” stating that the “NIH will not fund any use of gene-editing technologies in human embryos.” Federal funds are not allowed to support such research.

In February 2017, however, a committee for the National Academy of Sciences and the National Academy of Medicine condoned modifying embryos to correct mutations that cause “a serious disease or condition” when no “reasonable alternatives” exist.

And last summer, privately funded researchers in Oregon reported using CRISPR-Cas9 to correct a common mutation in fertilized human embryos.

“The questions surrounding eventual use of CRISPR-Cas9 in human embryos are not new,” says Lisa Parker, director of the University’s Center for Bioethics and Health Law. “They center on safety of the intervention and welfare of offspring, and on what is appropriate for parents to seek in (and for) their children and by what means…. We must also ask whether there will be equitable access to this technology, or if it will exacerbate health disparities.”

In any case, therapeutic applications are still “way into the future,” Tsang notes.

Zebra fish develop quickly. Scientists can inject an embryo, let it grow, and study the effects in just three days; yet the complexity of nature means that progress is slow.

“The operation (incorporating unnatural amino acids into an organism’s proteins) is surprisingly context sensitive,” says Deiters. “The scientists can’t always get a protein to express, and they don’t always know why. That unpredictability is a little frustrating, Deiters says, “but that’s why they call it research.”