



LUMINOSITY

Roger Tsien won a Nobel Prize for designing a rainbow of proteins that shine as they do their work within a cell. Now he's using his fine-tuned aesthetic to cure cancer and flag memory formation.

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ROGER TSIEN is ready to move beyond his signature accomplishments. Specifically, the Nobel-prize winning biochemist, who devised a way to use fluorescence to watch proteins in action, wants to change how cancer surgery is done, among other things. But a shift away from glowing proteins is turning out to be harder than he expected. “Maybe it’s foolish,” says Tsien, an HHMI investigator at the University of California (UC), San Diego. It is rather late in his career, he admits, to delve into an unfamiliar clinical problem. The impact of his work makes it easy to see why he feels like he’s swimming against the tide.

“There’s been this great transformation in biology because of the tools that Roger developed,” says HHMI investigator Susan Taylor, a biochemist at UC San Diego who helped recruit Tsien to the school. They have provided “enormous extra information” about how cells function, she says.


The 2008 Nobel Prize in Chemistry, which Tsien shared with Osamu Shimomura and Martin Chalfie, recognized his work

developing green fluorescent protein (GFP) markers to watch proteins in action. By manipulating genes from a glowing jellyfish and from corals, Tsien created a set of genes that produce colors ranging from violet to deep red. These genes allow scientists to tag any of the tens of thousands of proteins at work in the body to observe what they do, and, by marking multiple proteins, how they interact. GFP’s utility goes far beyond human biology as well; it’s been used to create bacteria that glow in the presence of arsenic, a significant problem in well water in Southeast Asia, and to identify explosives such as TNT.

Tsien isn’t leaving visualizing of otherwise invisible processes behind, but he’s set his sights on new targets: lighting up cancerous tumors for surgeons and watching memories form. His results on both fronts are promising. At a recent lecture at a Midwestern university, however, when he described his progress, the first question from the audience was about the work he’s been trying to leave behind: “When are you going to devise infrared fluorescent proteins?”

CELLULAR VISION

At a November science meeting at HHMI’s Janelia Farm Research Campus, Loren Looger wanted to set the stage for a discussion of his own research on sensors to study molecular



activity in the brain. So the *Janelia Farm* group leader described Tsien's early work, which focused on calcium.

Calcium is an important cellular messenger that controls neurotransmitter release from neurons, governs muscle cell contraction, and plays a role in fertilization. When Tsien was a graduate student at the University of Cambridge, researchers could measure calcium levels in only a few extremely large cells that could tolerate being poked with a needle. During his graduate and postdoctoral studies, Tsien developed fluorescent dyes to measure calcium in most animal cells without having to inject them.

Later that evening, Tsien gave his own presentation, a last-minute addition to the agenda at HHMI's request, to celebrate his Nobel win. Tsien himself was physically unassuming. Many of the slides were black and white bullet points put together in *Janelia's* library that afternoon. The presentation's color came from Tsien's passion for his work, a sense of humor about himself, and an interest in others. He explained that he'd decided to speak primarily to the graduate students in the audience.

"I wish to explain how you can screw up and be screwed up and still, some day, accomplish something," he began.

The first step? "Identify a big problem in biology, preferably one whose solution would assuage a personal sense of inadequacy," he said. Tsien, who grew up in Livingston, New Jersey,

comes from a family of high-achieving engineers—his father was a mechanical engineer and his mother's brothers were engineering professors at the Massachusetts Institute of Technology (MIT). Tsien's brother Richard, originally an electrical engineering major at MIT, is now a prominent neurobiologist at Stanford University and former member of HHMI's scientific review board.

Roger Tsien says that it was a given he'd end up in science, too, but his path to chemistry and biochemistry was in part shaped by his role as the youngest of three brothers in search of his own niche. "Part of the reason [I pursued] chemistry is that it was one of the things that Dad and my brothers didn't really like," he says. "Dad's idea of taking care of the lawn—since he was an impatient mechanical engineer—was to get on his hands and knees and dig up the weeds. I was allergic to them and wanted to pour herbicide on them from a distance."

Tsien explained that his work inventing calcium dyes at Cambridge was the fourth project within his dissertation; his first three efforts had run into dead ends. When he started work on the calcium indicator he didn't tell his advisor, who surely would have vetoed the effort. The subterfuge paid off, and his career was launched. Today, researchers around the world rely on the dyes Tsien created to study calcium, pH, sodium, and other molecules.

At the *Janelia* lecture, he showed an image sequence called the *Blush of Conception*. When a sperm fertilizes an egg, it initiates a wave of changes inside the cell, including creating an intracellular wave of calcium. Tsien made those processes visible. As the sperm hit the egg, the calcium change was depicted as a wave of color—as if the egg were blushing—washing across it from the point of contact.

Mark Ellisman, a professor of neurosciences and bioengineering at UC San Diego, notes that each time Tsien develops a new tool, in the paper that introduces it he affirms its value by showing how it can be used to understand some substantive problem in biology. "As soon as that's out, the world picks up on it and starts to use the technology on a large number of problems," says Ellisman, who collaborates so closely with Tsien that the two sometimes hold joint lab meetings.

"He's passionate about things that are useful to society," Ellisman adds. "He's got a very beautiful orientation to what's important."

BEYOND THE JELLYFISH

As a young faculty member at the University of California, Berkeley, Tsien's personal insecurity drove his next big success. Surely, he thought, the prestigious biochemists and molecular biologists at Berkeley were looking down their noses at a scientist in the physiology department working on a "mere metal ion like

calcium.” So he decided to find a way to use a process called FRET to build indicators of the essential cellular messenger cyclic AMP (cAMP).

When two fluorescent dyes get close to each other, one steals energy from the other, changing the color it emits. The process—called fluorescence resonance energy transfer, or FRET—is particularly useful in watching cAMP split apart the subunits of protein kinases that depend on cAMP.

Tsien began collaborating with Susan Taylor at UC San Diego, the world’s expert on cAMP-dependent protein kinase. But sending samples by FedEx between Berkeley and San Diego wasn’t working, so Tsien moved south.

“Even after the move, it took a year of my people being physically in the lab adjacent to Susan’s for us to make the cAMP reporter work,” Tsien says. “It required laboriously growing bacteria, purifying two proteins, chemically labeling them in a test tube with two different colors, recombining and repurifying them, and then microinjecting them into the cells of interest”—and the cells had to be large enough to handle microinjection.

Tsien fretted over these limitations. There had to be an easier way.

With transgenic technologies, he could insert foreign genes into a cell’s genome, making the cell create whatever protein the gene called for. Tsien searched for a gene that encoded a fluorescent protein he could monitor—a daunting challenge. He remembered reading a review of a protein called aequorin, which is produced by the *Aequorea victoria* jellyfish in the Puget Sound. “[The paper] said if you’re really careful, you can get rid of this awful contaminant, green fluorescent protein,” Tsien recalls. “In despair, I typed green fluorescent protein into Medline in early 1992.”

The rest, as they say, is history. He found that a scientist at the Woods Hole Oceanographic Institution, Douglas Prasher, had just cloned the gene for GFP. When Tsien contacted him, Prasher said his funding was ending and he was stopping work on it, but he

agreed to send the DNA for GFP to Tsien. Another scientist, Martin Chalfie also contacted Prasher and received the DNA.

Would the gene only work in the jellyfish? If GFP needed to interact with other chemicals in jellyfish cells in order to glow, it wouldn’t solve Tsien’s problem; he needed a protein he could add to any cell. Chalfie quickly answered that question by creating transgenic bacteria and worms that glowed, showing GFP would glow in other organisms as well.

Tsien immediately recognized the potential of GFP, but he also saw its flaws—it gave a big peak in the UV spectrum where scientists didn’t want to illuminate; the visible blue-green peak was much smaller. And in its native state, five-sixths of it was in a form useless to researchers. He modified the gene, creating a series of highly effective fluorescent proteins in colors from blue to red.

And that Midwestern questioner will be happy to know Tsien’s lab has made progress toward producing an infrared fluorescent protein for viewing cells and organs inside living animals without having to open the animals. (The deep red color of blood obscures the current palette of fluorescent proteins.)

The Nobel Prize can only be split three ways, but Tsien has been adamant about recognizing the contribution of Prasher, who eventually left science and now lives in Huntsville, Alabama.

“If there’s anyone who’s underappreciated, it’s Douglas Prasher,” Tsien says. “If he hadn’t cloned the GFP gene, progress in the field would have been delayed indefinitely. I don’t know anyone else who was working on the cloning.” Tsien and Chalfie flew Prasher and his wife Virginia Eckenrode to Sweden for the Nobel ceremony.

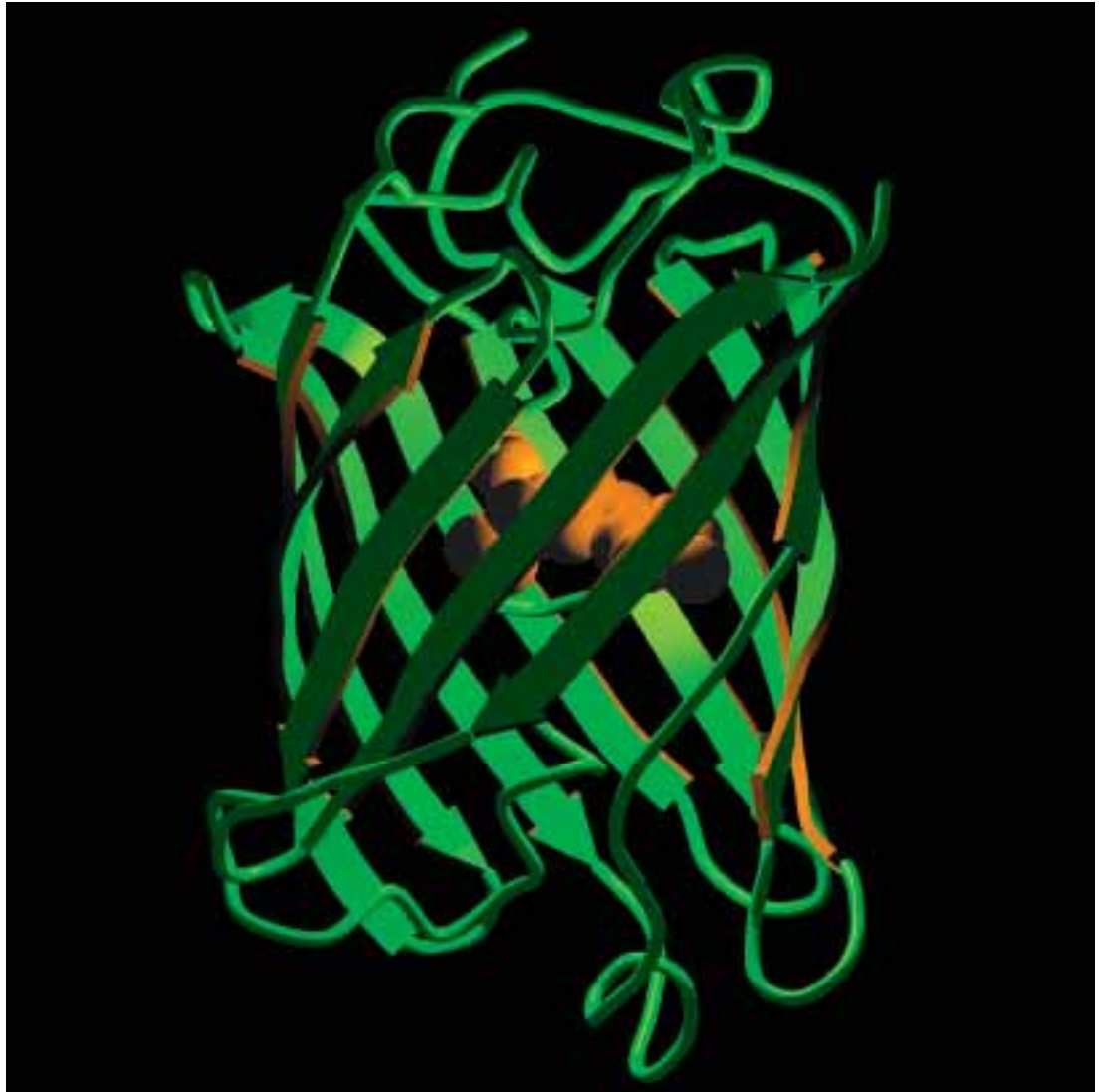
MARKER FOR MEMORY

“Seeing things, that’s sort of buried in my psychic makeup,” says Tsien, explaining why his work is so focused on visualization. Today, he’s applying that inner aesthetic to new problems.

“How and where synapses adapt to create and preserve a memory is one of the most important problems in neurobiology right now,” says Michael Lin, a postdoctoral researcher in Tsien’s lab. When new memories form, unique neural connections, or synapses, grow in size. This process involves new protein production, so Lin and Tsien are working on ways to watch the proteins that are created during synapse growth.

To do this, Lin fuses three genes in sequence: an easy to visualize tag, a protease from hepatitis C virus, and a synaptic protein of interest. The hepatitis viral protease likes to cut off anything attached to its own ends, so, left alone, the triple protein splits itself into its three constituents, leaving the synaptic protein untagged. If Lin administers an anti-hepatitis C drug that cripples the protease, all the fusion protein molecules made after that point remain intact with their tags still attached.

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THE BARREL-SHAPED GREEN FLUORESCENT PROTEIN, GFP, WAS FIRST ISOLATED FROM A GLOWING JELLYFISH.

The work remains at an early stage, but Lin has used the system to create transgenic flies, labeling a protein that's known to be a major structural component of synapses. Neither he nor Tsien thinks they've found the perfect marker for memory formation, but, says Lin, "We have a good enough candidate to begin work."

Their "dream experiment" would be to find a master protein that is freshly made when a new synapse is born but is not being continuously refreshed in existing synapses. They would then create an animal with that protein labeled and train it in some task while giving it the anti-hepatitis C drug. All copies of the master protein formed from that point forward would be labeled. By observing where those proteins accumulate, they could see where nerve synapses had formed or expanded. From a practical standpoint this process is a bit more daunting than it sounds. They still don't know all the proteins that help create synapses. Tsien hopes other scientists will take up the memory question as well, so that they can divvy up the many potential protein candidates.

GLOWING TUMORS

The effort that Tsien calls "foolish," but is taking most of his energy, is his foray into cancer. It reflects a wish to do something

about the disease that claimed the lives of his father and his Ph.D. supervisor.

When surgeons remove a tumor, they rely on their knowledge of how cancer tissues look and feel to remove as much as they can without taking out too much surrounding tissue. "The way we try to tell whether we have gotten it all is to take samples of what's left and send them to the pathology lab," says Quyen Nguyen, a head and neck surgeon at the UC San Diego School of Medicine who also works in Tsien's lab. Examining tissue for cancer can take 10 or 20 minutes per sample, so that process can mean keeping a patient under anesthesia for an extra hour or longer.

Fluorescent proteins don't help here, because their main advantage is that they can be delivered by gene transfer, which is fine for experimental animals but not human patients. Nguyen and Tsien have found a way to load tumors with synthetic nanoparticles that not only are visible by magnetic resonance imaging but also glow on the operating table, making the edge of the tumor visible to the surgeon. The technology holds promise as a way to detect tumors and improve the success of surgery and, possibly someday, as a way to deliver drug treatment.

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These nanoparticles are the molecular equivalents of self-adhesive name tags that come with a nonstick backing paper. As long as the “backing paper” is covering the “adhesive,” the molecules don’t stick to cells. Malignant tumor cells secrete specific proteases that they use to chew their way through normal tissues to reach the blood and distant organs. The “backing paper” is carefully designed to fall off when chewed by the tumor cells’ proteases, thus exposing the “adhesive” and coating the tumor cells with the nanoparticles.

The nanoparticles carry fluorescent and magnetic tags to make the tumors visible. Eventually, they may also carry drugs to kill any cancer cells left over after surgery. One obstacle is that other enzymes in a normal liver can also cut off the “backing paper” as currently designed, so that the liver picks up a lot of the nanoparticles. That doesn’t matter for surgery, but it could be a problem for delivering cell-killing drugs.

In transgenic mice with an especially aggressive form of breast cancer, surgical removal of the tumor offers only a 10 percent chance of tumor-free survival. But when Nguyen and Tsien made them glow, tumors were visually distinguishable from healthy tissue; tumor removal was more complete, and tumor-free survival quadrupled to 40 percent.

“[Tsien] can visualize, almost anthropomorphize, molecules in a very astute way, so that things work,” explains Nguyen. “He really has a sense of how these molecules interact.”

Their results, while not yet published, appear to bear out Nguyen’s praise. In addition to helping the mice live longer, they’ve tested the technique on biopsied human breast cancer tissue (results they plan to publish with the mouse studies); the cancer cells take up the marker. But, at least for now, mice are the only animals to benefit on the operating table. (As a side project, Tsien and Nguyen are working on a way to make nerves visible during surgery to help surgeons avoid damaging them.)

Between the lab and human trials, Tsien says, “there’s something called the valley of death, the gap between promising results obtainable with research funding versus the much more expensive studies necessary to convince companies to invest. Right now I’m looking at that valley of death, which is particularly wide during economic gloom.” He expects that clinical trials to test the safety and effectiveness of the technique in humans will cost millions of dollars.

But the potential payoff is enough to motivate this leap into new territory. “Some of the biggest touted therapies cost hundreds of

thousands of dollars per patient treated, and they buy you a few extra months and then you die anyway,” he says. If, for even a few patients who need surgery, he can help surgeons cut out all the cancer, he could buy those patients extra years.

What’s Important

That kind of motivation is what Tsien thinks should drive scientific inquiry. At Janelia Farm, fresh from notice of his Nobel, Tsien admonished the students that if they wanted a successful career in science, they shouldn’t be “unduly motivated or impressed” by prizes.

What they should do, he said, is explore research questions that give some form of day-to-day pleasure. They’ll need that interest to sustain them through the periods between big discoveries or the times when things just aren’t working, when they find themselves staring at their own valley of death.

He acknowledged, however, that there are some perks to a Nobel win. “Writing for samples is easier now,” he smiled. “People who used to ignore my e-mails are more responsive.” ■

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Correction: In a November 2008 article on funding for the Marine Biological Laboratory (MBL) in Woods Hole, Massachusetts, the *Bulletin* incorrectly stated that MBL has been offering courses since 1982. The correct date is 1892. The *Bulletin* regrets the error.