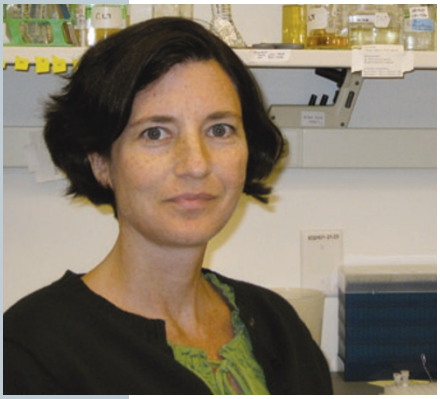
THE AUTHOR FILE

Chandra Tucker

Blue light pulls proteins together.

In a literature search a few years ago, Chandra Tucker came across an off-topic paper that intrigued her. It described a way to fuse plant proteins to a yeast transcription factor so that gene expression could be turned on and off with light. "It was really exciting; it was the first I'd heard of photoreceptors being used this way," she says.

Tucker, a biologist at Duke University, thought she could apply the technique more broadly, and she already had experience with light-sensitive proteins. Though she had never worked with plant photoreceptors before, her graduate work had involved their counterparts in mammalian eyes; her experiments required night-vision goggles along with other inconveniences. "I know how to work with proteins in the dark," she says.



Chandra Tucker, Duke University

As a postdoc in Stan Fields' laboratory at the University of Washington, Seattle, Tucker had already engineered a system in which small molecules could be used to stabilize proteins and restore their activity, but chemical systems have inherent limits, she says. Small molecules take time to diffuse and do not allow for precise spatial resolution. In contrast, light can be instantly trained onto specific cells or even specific regions of cells.

Tucker discussed her idea with her husband, coauthor Matthew Kennedy, a postdoc in Michael Ehlers' lab who works on imaging techniques to monitor neural plasticity. He was enthusiastic about the potential of the idea for use in neurons, where many localized events occur on a fast timescale.

The idea was that two plant proteins that interact in response to light could be used to dimerize proteins, forcing them to desired cellular locations. But the plant proteins Tucker had read about (phytochrome *b* and PIF3) did not perform as she had hoped. In addition, the two proteins had some affinity for each other even in the dark and required a cofactor that had to be supplied to yeast and animal cells. "There were many reasons why this system wasn't ideal," Tucker says, "the main one being that it wasn't working for us."

Another paper identified a light-dependent interaction that seemed more promising. A protein called cryptochrome 2 interacted with its binding partner under blue light and used cofactors that are naturally present in nearly all cells. To test the system, Tucker first verified the original results in yeast, observing the blue light–dependent interaction that had originally been reported. Tucker then designed mammalian expression vectors that attached a red fluorescent protein to cryptochrome 2 and green fluorescent protein to its binding partner and gave them to her husband to image.

Despite what she had seen in yeast, Tucker did not necessarily expect these initial constructs to work. Yeast and mammal cells come from different kingdoms, after all, and her experience with phytochrome had prepared her for a long slog. But Kennedy called her at the lab a couple of hours later. "He was very excited that it worked, and it was super robust." Bright yellow points formed under blue light, a clear indication the green and red proteins had been brought together. They dissociated after a few minutes in the dark and reassociated in half a second when the blue light was turned on again. "It wasn't a case of 'maybe it's working; let's try it again,'" Tucker says.

> "There are so many applications, it's going to be hard to focus," —Chandra Tucker

In addition to yeast cells and HEK293 cells, the system also worked in slices of mouse hippocampus. Moreover, the system works with two-photon microscopy as well as with less-sophisticated equipment. For some applications, researchers in Tucker's lab simply use an LED light commonly sold for seasonal affective disorder.

Tucker and coauthors split the transcription factor GAL4 and a DNA recombinase and showed that these could also be made functional by light; gene expression could be controlled precisely with the 'dose' of light administered. The strategy should work for a wide range of proteins, she says. "In theory, any protein can be split."

This system is one of several recently published techniques that uses light to manipulate proteins. In addition to pulling split proteins together, Tucker thinks her system might be used to sequester proteins or render them inactive. And precisely timed flashes of light should allow researchers to titrate the extent of protein function, allowing quantitative analysis, she says. "There are so many applications, it's going to be hard to focus."

Monya Baker

Kennedy, M.J. *et al.* Rapid blue-light-mediated induction of protein interactions in living cells. *Nat. Methods* **7**, 973–975 (2010).