Adam Cohen: Visualizing cellular voltage

Cohen uses rhodopsins as optical sensors of cellular voltage.

When ion concentrations differ on either side of a membrane, this produces a difference in electrical potential, or voltage, across the membrane. Neurons utilize this voltage to transmit information in the form of electrical signals. But might cell voltage also affect processes such as embryonic development or the behavior of yeast and other microbes? These questions cannot be answered using the traditional tools of electrophysiology.

Physicist Adam Cohen is interested in viewing biological problems in new ways (1, 2) and lately has been working to make it possible to study the roles of cellular voltage. Cohen recently discovered that some light-sensing proteins of the rhodopsin family can be "run in reverse" so that, instead of changing a cell's voltage in response to light, they fluoresce in response to changes in cellular voltage (3–5). Cohen's group has since worked to optimize these new optical voltage sensors and has great plans for this new technology, as he told us when we called him at his Harvard laboratory.

SCIENCE ABROAD

Did you have any role models growing up?

My father is a professor at Rockefeller University, and he and my mother were very supportive of my interest in science. They gave me an oscilloscope for my bar mitzvah because they knew I loved tinkering with electronics.

I was also strongly influenced by one of my science teachers, the advisor to my high school's science club. He fostered my interest in science, but he and I also talked a lot about the country he came from, Liberia. He told me a lot about what was going on there and inspired me to get involved with Liberian science education.

Have you been to Liberia?

Yes, twice. The country's scientific infrastructure had been largely destroyed during a 14-year-long civil war that ended in 2003. Worse, most of the teachers had been either killed or forced to flee the country, so Liberia had to rebuild its scientific systems basically from scratch.

My second trip to Liberia was made with a woman named Liz Wood and a friend of mine from high school, Ben Rapoport, with the aim of helping restore science education in Liberia. We worked with the faculty at the University of Liberia to develop a science curriculum. It included lectures and research projects that could be done with materials available in the Liberian marketplace so they wouldn't have to import costly foreign materials. I would love to go back there, but I have to devote a lot of time to my work right now. So instead I help organize trips for other groups that want to go.

DOUBLING DOWN

You did two PhDs…

Yes. My first one was in Cambridge, UK. I worked on several projects there that all involved theoretical physics. My main project was on the theory of light–matter interactions and was done with a scientist back in the US, Shaul Mukamel.

> "I said to myself, 'Can we run [rhodopsins] in reverse?'"

But I had really wanted to do experimental work since first experiencing it as an undergraduate at Harvard, so in my second year at Cambridge I decided to come back to the US to start over on my PhD. I wasn't expecting to get a PhD from Cambridge, but some of the people I worked with there suggested that I submit my work. So I did. Then I was in the bizarre position of being a first-year grad student at Stanford with a PhD from Cambridge. I worked with W.E. Moerner on methods for trapping and manipulating single molecules under a microscope, and after I graduated I spent six months as a postdoc with W.E. before starting my own lab at Harvard.

PHOTO COURTESY OF STU ROSNER

Adam Cohen

What kind of problems did you envision your lab working on?

We explored a huge variety of different projects before things started to stick. The unifying theme of our projects was that they all used sophisticated optical setups involving microscopy and lasers. But we didn't do anything with cells for the first few years because I didn't know anything about cell biology. When my lab became interested in working with cells, I had to go sit in on undergraduate biology courses to pick up the basics.

How did you become interested in cell biological questions?

I gave a talk at Boston University where I met Kenneth Rothschild. He told me about these interesting proteins called microbial rhodopsins that microorganisms use to convert sunlight into energy or to sense sunlight so they can migrate towards or away from it. We started studying these proteins in the lab, and for about two years we worked on developing an optical trick to observe the conformational changes that allow these proteins to sense and respond to sunlight. But the amount of light that we had to shine on the system to see the signal from a single molecule was more than the protein would ever see

Text and Interview by Caitlin Sedwick
csedwick@gmail.com

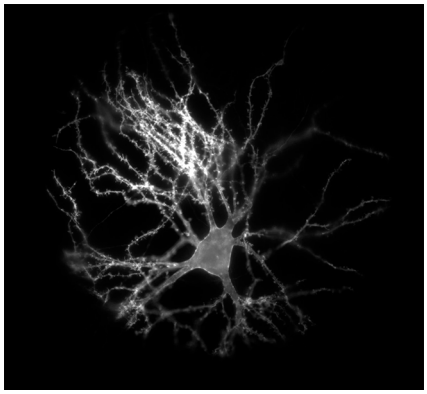

IMAGE COURTESY OF SAAMI FARHI

Rat hippocampal neurons expressing a genetically encoded fluorescent voltage indicator.

in nature. It was so intense that it basically fried the molecule.

By that point we had sunk two years of work into this protein, and I was trying to find some way to salvage all that effort. I had a vague notion that neuroscientists were interested in visualizing electrical activity in neurons. And here we had these proteins that absorb sunlight, and some of them convert that energy into a voltage across the cell membrane. So I said to myself, "Can we run these things in reverse?" Instead of having light come in and a voltage come out, can we use a change in voltage to produce a detectable optical signal?

I got in touch with Joel Kralj, who was a grad student in Ken Rothschild's lab, and asked him if he wanted to try to turn this very vague, somewhat crackpot scheme into reality for a postdoc project. Amazingly enough, he said yes.

DIRECTED EVOLUTION

You first got this to work in bacteria…

Joel had been expressing mutated rhodopsins in bacteria for a few months and had occasionally taken photos showing fluorescence coming from the bacteria. Then one day I suggested he try taking a movie, so he did that, and it showed the bacteria were all blinking on and off. This was a huge surprise to us.

We spent about a year exploring this, trying to figure out what was going on. It turns out that bacteria generate electrical spikes, a little bit like action potentials in a neuron. It was known that bacteria express ion channels, but nobody had ever observed electrical behavior in individual bacteria because the cells are too small to get an electrode into. We're still interested in this phenomenon, but my whole lab has been seduced by eukaryotes. The bacterial work is on hiatus.

So you're a physicist with a tissue culture hood?

Not only a tissue culture hood but also a mouse colony. We also have human stem cells and live zebrafish. We've gone whole-hog biology. [Laughs]

How did you get this to work in eukaryotes?

The bacterial work was extremely encouraging because it showed that we did have a voltage indicator, and it seemed like its sensitivity and speed were vastly superior to anything that anybody had made before. So I thought, "Great, let's put the gene in a mammalian vector, learn how to culture mammalian cells, and look in neurons."

"We've gone whole-hog biology."

So we did that, and it didn't work. The protein was expressed, but it didn't traffic to the plasma membrane, which was where it needed to be to act as a voltage sensor. A postdoc, Adam Douglass, in the lab of our collaborator Florian Engert, made 45 different constructs, and for a year we tried everything we could think of to get it to traffic to the plasma membrane. Nothing worked, but then, just as I was thinking about giving up, Ed Boyden's lab published a paper in which they tested many different homologous rhodopsins from different species and found several that worked well in the forward direction, converting light to voltage. I thought, "What are the odds that the bacterial protein we were working on was the only one of the thousands of proteins in this family that can show voltage-sensitive fluorescence?" So we took the best protein from Ed's paper, Arch, and expressed that in mammalian cells. It worked on the first try. We've since worked with Robert Campbell's lab at the University of Alberta to optimize the protein, making it brighter and faster and ensuring that it doesn't pass a proton current.

Where are you taking this next?

I'm very interested in the diversity of bioelectric phenomena in nature. Every cell has a membrane around it, and there are loads of systems—yeast, plants, and mitochondria, for example—where for various reasons it hasn't been possible to measure the voltage but where membrane voltage may affect cell behavior. Our modified rhodopsins can let us ask lots of really interesting questions about these systems.

I'm also working closely with stem cell biologist Kevin Eggan to express these proteins in human stem cell–derived neurons and cardiomyocytes with the idea that we can use rhodopsins to study the electrophysiology of these cells with a throughput that you could never get with manual patch-clamp measurements. There are many medical applications for such technology—it could be used to monitor new drugs' cardiotoxicity or neurotoxicity, for example—and Kevin and I have recently founded a company to explore its more commercial applications.

1. Cohen, A.E., and W.E. Moerner. 2006. *Proc. Natl. Acad. Sci. USA.* 103:4362–4365.
2. Elting, M.W. et al. 2013. *Opt. Express.* 21:1189–1202.
3. Kralj, J.M. et al. 2011. *Science.* 333:345–348.
4. Kralj, J.M. et al. 2012. *Nat. Methods.* 9:90–95.
5. Hou, J.H., V. Venkatachalam, and A.E. Cohen. 2014. *Biophys. J.* 106:639–648.

PHOTO COURTESY OF BEN RAPOPORT

Cohen teaching a science class at Booker Washington Institute, Kakata, Liberia.