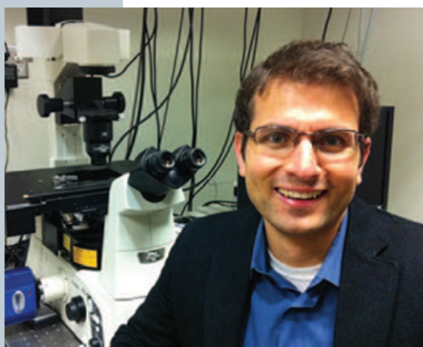
THE AUTHOR FILE

Khalid Salaita

Measuring single-molecule forces with light.

Starting a new lab is a bit like winning the lottery, says Khalid Salaita, who joined the faculty at Emory University in Atlanta in 2009. "It's sort of like someone handing you a million dollars and saying, 'What's your dream question'?"



Khalid Salaita

For Salaita, that dream has long been science. As a boy growing up in Amman, Jordan, he spent afternoons watching dubbed versions of *Beyond 2000*, a British television show about technologies expected to improve life in the twenty-first century. It made a lasting impression.

Salaita began his graduate studies with nanotechnologist Chad Mirkin at Northwestern University in Illinois, working out ways to use an atomic force microscope to deposit molecules on a surface. He liked developing tools, but he was hungry for open-ended questions. "I wanted to move into biological systems because that's where all the good questions are," he says. As a postdoc with Jay Groves at the University of California, Berkeley, Salaita used surface chemistry to study the spatial distribution of a receptor tyrosine kinase on cell membranes and found striking correlations with cancer cells' invasive behavior.

After establishing his own lab, Salaita decided to study the spatial distribution of another cell-surface protein, the epidermal growth factor receptor (EGFR), which is at the apex of a cell-signaling pathway implicated in many cancers. But as he began to discuss preliminary results with colleagues in other departments, he found that biologists were often less interested in how EGFR molecules were clustering on the surface of the cell than in how clustered EGFR molecules were moving into cells with their bound ligands. "A surface chemist doesn't generally think of internalization," Salaita reflects, "but if you're a biologist, that's one of the first steps of the signaling pathway."

He began looking into ways to study forces at the membrane, which would be necessary for internalization. "It became clear that we were working on something that was not already out there," Salaita says.

The lab was already using fluorescence microscopy, and Salaita thought there might be a way to develop sensors based on fluorescence resonance energy transfer (FRET) by pairing a donor fluorophore with a non-fluorescent acceptor molecule that quenches fluorescence, and using this interaction to determine the distance between the molecules. A 'stretchy' sensor is attached to a surface coated with quencher molecules at one end. The other end is attached to a ligand modified with a donor fluorophore. As the ligand is pulled into a cell, the donor is tugged away from its quencher, and increased fluorescence can be measured, revealing the force the receptor exerts on the ligand.

The learning curve was steep. Salaita's graduate students had been trained in synthetic chemistry, but they had not done surface modification, cell culture or quantitative FRET, a finicky technique. "We lost lots of cells to infection," recalls Salaita, but they expanded their skills and kept on working.

Eventually, the team found that a sensor made of polyethylene glycol offered the appropriate length and the right combination of flexibility and rigidity. It could be chemically attached to the ligand at one end and to the small molecule biotin at the other end, which allows the sensor to be fixed to a streptavidin-studded surface. Salaita thinks people who may have been intimidated by other techniques for measuring forces, such as atomic force microscopy or force spectroscopy, may find this one more accessible. "Anyone with a microscope can do it," he says.

As Salaita's team was developing its sensor, other groups described how proteins expected to experience forces can be re-engineered into force sensors with the addition of two fluorescent proteins linked by a 'springy' domain. That's powerful, says Salaita, but many proteins, particularly membrane proteins, will lose their activity with that kind of alteration, and his tethered-ligand approach works with unmodified cells, such as those acquired from a biopsy.

Salaita has already been approached by potential collaborators, and a commercial reagent supplier is considering producing assay kits consisting of surfaces studded with sensors attached to a ligand of interest. Meanwhile, Salaita is exploring additional ways to use the technique. "People have ways to measure the forces of cells moving," he says. "We have a way to see what *molecules* are feeling this tension." Another possibility is investigating properties in which a ligand can only activate its receptor if attached to the extracellular matrix, or even to another cell.

Starting from a dream question has taken Salaita's lab to questions he hadn't imagined. "This was a couple scribbles on a notepad a couple years ago," he says. "It works much better than we thought it would."

Monya Baker

Stabley, D.R., Jurchenko, C., Marshall, S.S. & Salaita, K.S. Visualizing mechanical tension across membrane receptors with a fluorescent sensor. *Nat. Methods* **9**, 64–67 (2012).

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