Chemists reveal the force within you

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A new method for visualizing mechanical forces on the surface of a cell, reported in *Nature Methods*, provides the first detailed view of those forces, as they occur in real-time.

"Now we're able to measure something that's never been measured before: The force that one molecule applies to another molecule across the entire surface of a living cell, and as this cell moves and goes about its normal processes," says Khalid Salaita, assistant professor of biomolecular chemistry at Emory University. "And we can visualize these forces in a time-lapsed movie."

Salaita developed the florescent-sensor technique with chemistry graduate students Daniel Stabley and Carol Jurchenko, and undergraduate senior Stephen Marshall.

"Cells are constantly tugging and pushing on their surroundings, and they can even communicate with one another using mechanics," Salaita says. "One way that cells use forces is evident from the characteristic architecture of tissue, like a lung or a heart. If we want to really understand cells and how they work, we have to understand cell mechanics at a molecular level. The first step is to measure the tension applied to specific receptors on the cell surface."

The researchers demonstrated their technique on the epidermal growth factor receptor (EGFR), one of the most studied cellular signaling pathways. They mapped the mechanical strain exerted by EGFR during the early stages of endocytosis, when the protein receptor of a cell takes

in a ligand, or binding molecule. The results showed that the cell does not passively absorb the ligand, but physically pulls it inside during the process. Their experiments provide the first direct evidence that force is exerted during endocytosis.

Mapping such forces may help to diagnose and treat diseases related to cellular mechanics. Cancer cells, for instance, move differently from normal cells, and it is unclear whether that difference is a cause or an effect of the disease.

"It's known that if EGFR is over-active, that can lead to cancer," Salaita says. "And one of the ways that EGFR is activated is by binding its ligand and taking it in. So if we can understand how tugging on EGFR force changes the pathway, and whether it plays a role in cancer, it might be possible to design drugs that target this pulling process."

Several methods have been developed in recent years to try to study the mechanics of cellular forces, but they have major limitations.

One genetic engineering approach requires splitting open and modifying proteins of a cell. This invasive technique may change the behavior of the cell, skewing the results.

The technique developed at Emory is non-invasive, does not modify the cell, and can be done with a standard florescence microscope. A flexible polymer is chemically modified at both ends. One end gets a florescence-based turn-on sensor that will bind to a receptor on the cell surface. The other end is chemically anchored to a microscope slide and a molecule that quenches fluorescence.

"Once a force is applied to the polymer, it stretches out," Salaita explains. "And as it extends, the distance from the quencher increases and the fluorescent signal turns on and grows brighter. We can determine

the force being exerted by measuring the amount of fluorescent light emitted."

The forces of any individual protein or molecule on the cell surface can be measured using the technique, at far higher spatial and temporal resolutions than was previously possible.

Many mysteries beyond the biology and chemistry of cells may be explained through measuring cellular forces. How does a cancer cell crawl when a tumor spreads? What are the forces involved in cell division and immune response? What are the mechanics that allow groups of cardiac cells to beat in unison?

"Our method can be applied to nearly any receptor, opening the door to rapidly studying chemical and mechanical interactions across the thousands of membrane-bound receptors on the surface of virtually any cell type," Salaita says. "We hope that measuring cellular forces could then become part of the standard repertoire of biochemical techniques that scientists use to study living systems."

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