# Single-molecule imaging reveals how cells prepare to interact with the world

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Researchers at Harvard Medical School have discovered that structural elements in the cell play a crucial role in organizing the motion of cell-surface receptors, proteins that enable cells to receive signals from other parts of the organism.

This discovery, published in the Aug. 19 issue of the journal *Cell,* fills a fundamental gap in the understanding of how cells relate to biochemical signals, including pharmaceuticals, and could have profound implications for drug development and the treatment of cancer and other diseases.

The findings are already prompting the design of a new lecture on cell signaling in one basic biochemistry course at HMS.

"We found that the way the receptors are organized in the membrane and the way they move around are controlled by the cytoskeleton," said Khuloud Jaqaman, instructor in the Department of Systems Biology at HMS and first author of the study. This dynamic organization promotes signaling function by encouraging receptors to cluster, even if briefly, she said.

Jaqaman and Gaudenz Danuser, HMS professor of cell biology, working with Sergio Grinstein from the Hospital for Sick Children in Toronto as well as colleagues at the University of Alberta, Edmonton, studied the motion of CD36, a receptor in human macrophages, a type of white blood cell that plays a role in immune response. CD36 detects oxidized

LDL (oxLDL), a lipoprotein implicated in atherosclerosis.

Receptors are like the antennas in a cell's communication system with the world outside their membrane. The cytoskeleton, which includes a fine meshwork of actin fibers and an array of radiating microtubules, gives the cell its shape.

Like many receptors, CD36 can't work alone; a group of receptors must cluster together to send a signal into the cell. Until now, very little was known about how those functional groups of receptors formed. The cell and receptors were thought to wait "at rest" until a chemical signal happened to appear, causing receptors to coalesce.

This study reveals a much more dynamic "before" picture, with structures that precondition the cell to respond to signals. The researchers say that their work clearly demonstrates how "resting" receptor movements are functionally relevant to the transmission of signals into the cell.

Grinstein, a senior scientist at Toronto's Hospital for Sick Children whose interests include understanding how macrophages work, approached Danuser for imaging and analysis expertise. Grinstein wanted to study CD36 at the single-molecule level in live cells and in real time under a microscope.

Using an automated particle-tracking algorithm she had developed to overcome the challenges of imaging such minute, complex interactions, Jaqaman analyzed these single-molecule movies to dissect the receptor behavior and its regulation.

The movies reveal three kinds of motion by the receptors, which are sensitive to strands of the cytoskeleton's actin meshwork adjacent to the cell surface. As receptors roam about, they bump into these strands,

slowing, stopping or changing direction. Some wander freely about the surface of the cell. Others become temporarily stuck inside a pocket of the mesh, as if trapped in a cage. Finally, some of the receptors travel linear paths.

These paths follow elongated "corridors" alongside the cell's microtubules, another part of the cell's cytoskeleton, radiating in more-or-less straight lines from the nucleus.

How the corridors form remains a mystery. The researchers suspect that they emerge from interactions between microtubules and actin, which remove actin strands from the path of the receptors.

In these narrow corridors free of actin strands, receptors scurry to and fro with more freedom, regularly bumping into one another, forming clusters that stick together fleetingly and then drift apart.

The researches suspected that these pre-formed clusters aid in signaling, so to test that theory, they disrupted the cytoskeleton. Sure enough, when the corridors disappeared, the cell no longer responded effectively to oxLDL.

Jaqaman compares the receptors in linear paths to people in the hallway of an office building. "People in the hallway are much more likely to bump into and chat with colleagues than people who stay in their offices all day, like receptors trapped in actin cages, or people wandering around the city, like receptors wandering freely around the cell surface," she says.

Jaqaman and Danuser stress that the mechanical nature of these structures—and how they relate to the cell's chemical and mechanical environment—may be key to understanding how healthy and unhealthy cells function, replicate and grow. For example, it is well known, they

said, that tumors are mechanically stiffer than normal tissues. In one provocative scenario, Danuser speculates that the broad variation in the mechanical properties between cancer tissues in different patients may be a key reason for the variable success of cancer chemotherapies that target cell-surface receptors.

"While most current research focuses on the study of oncogenes and tumor suppressors, it might be just the intrinsic change in cancer-tissue mechanics that leads to the change of signaling," Danuser said. "I think we absolutely have to look into that."

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