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Cancer Cells Have Pull

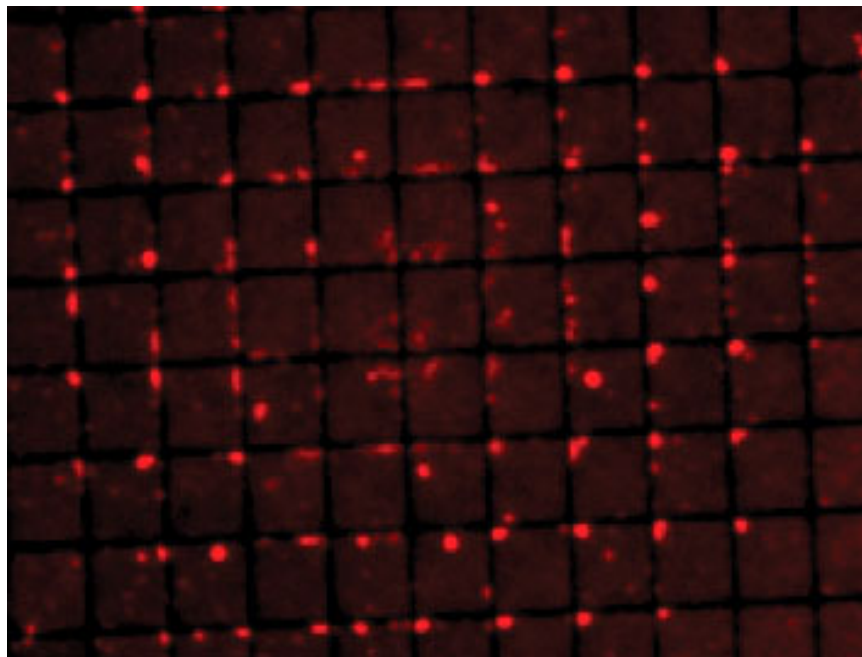


Image Title: Nanoscale patterns of metal on glass substrates govern the mobility of organic molecules, lipids and proteins, which form a coating 'supported' membrane. This synthetic cell surface can replace the surface of a natural cell and form an interface with a living cell. In this way, synthetically prepared nanometer scale patterns can be imposed on the living chemical processes in live cells, exposing new roles for spatial and mechanical effects in biology and disease. - Alex Smoligovets

Imagine that you have been asked to greet 26 of the world's most notorious criminals. Blindfolded and seated in a very narrow hallway, you meet them one by one. The criminals are clearly uncomfortable in such close quarters, and say nothing as each one takes you by the hand. You are then asked to rank the 26 according to how hard they pulled your hand. Matched against their rap sheets, your mechanical ratings reveal an unexpected pattern: the more violent the criminal, the harder they pulled your hand.

The "criminals" in this case were 26 well-studied mammary epithelial cancer cell lines. The blindfolded glad-hander was an artificial cell surface designed

to test how tightly cells bind to each other. The discovery that came out of the scenario -- that each tumor's aggressiveness is strongly correlated to its mechanical pulling power -- surprised the phantom cell's creator, biophysicist Jay T. Groves, a Howard Hughes Medical Institute investigator at the University of California, Berkeley and the Lawrence Berkeley National Laboratory.

Groves, who studies spatial organization by building half-biological, half-synthetic devices that can disrupt or rearrange life on the cell surface, says the team's discoveries point to a new role for spatial organization in living things. "Life is not just determined by what molecules are in a cell but where they are," he says. "There is essentially no chemical difference between being alive and being dead. The chemical content has not changed. The only thing that's changed is the organization."

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- Jay T. Groves

Groves's nanodevices are designed to pull apart and measure that spatial organization. He and his colleagues report on a series of experiments studying how mechanics and space affect cell-cell signaling in the March 11, 2010, issue of *Science*.

According to Groves, investigating these properties demands that researchers are comfortable crossing the boundaries of traditional scientific disciplines. Members of his lab happily mix techniques from materials science, physical chemistry, cell biology, and bioinformatics to collect and analyze data – a strategy he said was intentional from the start. "I saw questions and problems concerning how cells work that were outside the scope of classical cell biology and also inaccessible to physical scientists collaborating with biologists," he says. "These sorts of problems, of which our present work is a perfect example, require a new generation of students who speak both biology and physical science as native languages."

For the current study, Groves and his cross-disciplinary colleagues rigged an experimental platform that allowed them to manipulate the interface between a high-affinity receptor on the surface of cancer cells -- EphA2 -- and the ligand that binds to it, ephrin-A1. In the experiments, ephrin-A1, which normally resides on the surface of unsuspecting target cells, was instead protruding from Groves's artificial cell surface.

That phantom cell surface, created with techniques that Groves developed as a graduate student, is a supported lipid bilayer membrane that floats atop a glass coverslip plated with a grid pattern of chromium. The grid is cut by a process known as electron beam lithography. Metal plating the remaining structure divides the glass subsurface into corrals as small as 0.5 micrometers wide – much smaller than most animal cells. These corrals are separated by metal lines 10 nanometers high and 100 nanometers wide. (A nanometer is one billionth of a meter.) The differing properties of the metal and glass surface restrict the lateral movement of receptor-ligand complexes. By using such small structures, Groves' research team is able to reach inside of a single cell and begin to examine organization all the way down to molecular levels.

The researchers inserted ephrin-A1 ligands into the supported membrane and brought them into close contact with cancer cells presenting EphA2 receptors. "We were originally focused primarily on the role of spatial organization and molecular clustering as regulators of cell signaling processes," Groves says. "But as we looked at the cells, it was obvious that the EphA2 clusters were under tension. We could see them all pulled tight up against the boundaries of their respective nanofabricated corrals."

In the traditional view of cell-cell signaling, receptors bind ligands and form cluster complexes where the process of endocytosis, the drawing of outside material into the cell, begins. "If you read the literature, once the receptor binds the ligand, the next step is endocytosis. But that's not the next step," says Groves. "Something else happens in between. The cell pulls laterally on that cluster and moves it across the interface a distance of microns. It's that lateral tug that turned out to be most interesting because when we resisted that pull, the behavior of the cell changed."

Mechanical resistance in the form of unmovable grid walls stopped cells from a highly metastatic tumor line, MDA-MB-231, in their tracks. "These guys are very invasive," says Groves. "They are on a hair trigger and are going to invade, no matter what." But the nano-scale fences corralled MDA-MB-231, limiting its ability to pull together large clusters of receptor-ligand complexes in order to kick off the process. The experiment demonstrates that a physical factor alone mechanical resistance could change a tumor cell's behavior without altering its genetics, biochemistry, or chemical environment.

Looking beyond MDA-MB-231, the researchers turned to a library of 26 other breast cancer epithelial cell lines. They discovered that by using statistical analyses to compare the cells' pulling strength, they could neatly order the tumor lines by invasiveness.

The pulling comes from the protein motors, actin and myosin, that power the cell's cytoskeletal transport system. Groves believes the discovery that this cytoskeletal pulling power correlates with EphA2 receptor signaling and cancer invasiveness could be a vital clue into EphA2's well known -- but poorly understood -- role of in cancer metastasis.

Groves says that these discoveries point to a new role for spatial organization in living things. Classically, cellular signaling has been imagined as chemistry in a flask, he says. When cells click together, ligand and receptor molecules set off a cascade of chemical reactions that move step by step down a signaling pathway that changes the cell. But cells are not test tubes, says Groves, especially on the cell surface where location, force, and space are powerful determinants of how molecules come together.