# With record resolution and sensitivity, tool images how life organizes in a cell membrane

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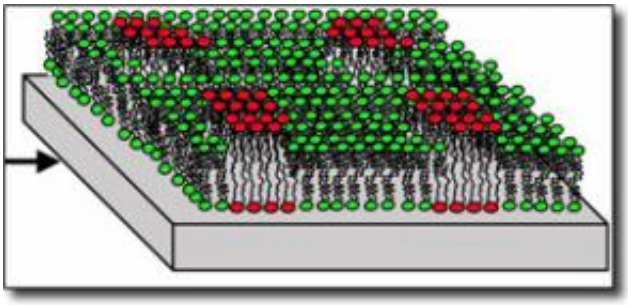


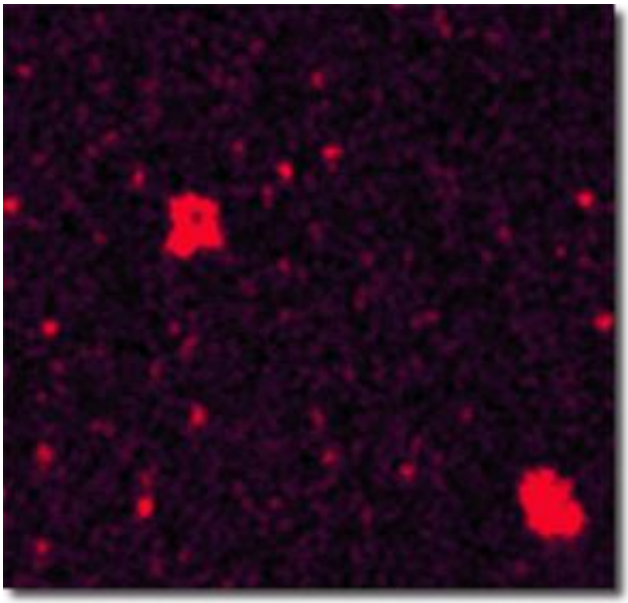
Researchers induced the formation of tiny lipid raft-like gel domains on silicon chips (above). They then used LLNL's high-resolution secondary ion mass spectrometer, the NanoSIMS, to image the samples and detect the gel domains at lateral dimensions as small as 100 nanometers (about 1,000 times smaller than the diameter of a human hair). The gel domains are shown as red in both the schematic and the NanoSIMS ion image (below). Credit: Lawrence Livermore National Laboratory

What's the difference between a lifeless sack of chemicals and a living cell? It's all in the way they're organized, according to Stanford biophysical chemist Steven Boxer. With colleagues at Stanford, the University of California-Davis and Lawrence Livermore National Laboratory, he has developed a way to image cell membranes with unprecedented resolution-on the order of 100 nanometers, a scale larger than individual molecules but much smaller than entire cells.

Understanding the chemical composition and organization of cell

membranes-what components reside next to each other, how many of each there are and how they respond to their environment-may reveal the secret lives of cells in both health and disease. The researchers report their findings in the Sept. 29 issue of the journal *Science*.

"The organization of components in biological membranes leads to function," Boxer, the Camille and Henry Dreyfus Professor in Chemistry, said in an interview in his office on campus. The Biophysical Society recently named Boxer one of five Fellows and praised his "seminal contributions and advancement of the field of biophysics through his groundbreaking research in several areas: supported membranes, Stark effect spectroscopy of proteins, properties of autofluorescent proteins and photosynthetic reaction centers."



Boxer's collaborators included chemistry postdoctoral fellow Mary Kraft

of Stanford, who isotopically labeled the lipids used in the experiments, prepared the samples and performed all the measurements, Associate Professor of Chemical Engineering and Materials Science Marjorie Longo at the University of California-Davis, whose earlier work on phase-separated membranes on surfaces inspired the system used in the work, and scientists Peter Weber and Ian Hutcheon, both experts in imaging mass spectrometry at the Lawrence Livermore National Laboratory (LLNL), who provided access to the state-of-the-art machine used in the experiments.

That machine is a highly specialized mass spectrometer, which analyzes the mass of small molecular ions formed when a focused ion beam runs across the surface of a sample. "You take everything in the beam's focal area, which is about 100 nanometers in diameter and about 10 nanometers deep for our experiment, and you obliterate it," Boxer said, explaining how the machine works. "Then you sample the fragments by mass spectrometry. Then you move over and you go another 100 nanometers and you obliterate everything. And now you see if what's in each 100 nanometer region is the same or different from the next region. And so you just raster this beam across the surface, and by rastering over and over and over again, you build an image."

Called NanoSIMS 50, the mass spectrometer is manufactured by Cameca Instruments of France and housed at LLNL. With a price tag of $3 million, it does a lot more than its cousins common in labs everywhere. It allows scientists to probe the composition of cell membranes with a higher resolution than light microscopy. By providing information about chemical composition of a sample, it fills a gap left by atomic force microscopy, which provides high-resolution information about topography, but not chemistry, as its microscope tip "feels" its way through samples. Plus it handles samples less ordered than those addressed by X-ray crystallography, which requires that samples be turned into crystals before analysis.

Geologists, cosmologists, materials scientists and engineers covet the machine for characterizing hard materials, such as rocks, space dust, polymers and nanoelectronics. But in the last five years, biologists have become interested in employing it to characterize components of biological samples on a length scale of 100 nanometers or less.

"There's currently no way to do that," Boxer said. "You can't get more multidisciplinary than something like this. This is a big deal in terms of an analytical method with very high sensitivity and high spatial resolution and high chemical information content."

Boxer's group used atomic force microscopy to locate interesting places and then employed the NanoSIMS 50 to determine what was there chemically. "Either technique by itself would be I think insufficient, but combined, they're really powerful," Boxer said. The combination of techniques allowed the researchers to distinguish debris from features of interest.

"The real point is that you can do quantitative analysis," Boxer said, emphasizing that this research allowed the first high-resolution mapping of chemical features in a region of interest. "We can analyze a few percent of one component in the presence of other components...It's exquisitely sensitive."

Sensitivity is important because cell membranes are not pure materials. "We're looking at mixtures of things, and we want to be able to say that we've got one molecule in 20 of type A mixed in with type B, or something like that," Boxer said.

## This world is flat-sometimes

"Imagine how a cell could divide if it weren't for the fact that the membranes were flexible," Boxer said. "They must be flexible. You have

endocytosis, exocytosis, all these processes which involve dynamic reorganizations of membranes."

The membrane that surrounds a cell or the organelles within cells is made of two layers of phospholipids-microscopic "lollipops" with water-loving (hydrophilic) heads that point outward and water-fearing (hydrophobic) tails that point inward. The cell membrane is studded with proteins like receptors and ion channels, which cross the membrane many times and transmit signals from the outside of the cell to the inside. Others don't cross the membrane at all-they are anchored to the membrane by lipid-like entities or by single transmembrane helices. The membrane is a fluid and components can move around laterally to differing degrees, like swimmers in a pool. The NanoSIMS 50 lets scientists determine the locations of specific swimmers and count how many swimmers are in the pool.

What scientists really want to know is what membrane components are near what other components, how that organization changes over time and how that organization leads to the emergence of function, Boxer said.

In their experiment, the scientists analyzed a model system to reveal its lateral organization. First they used lipids with either "short" or "tall" chemical structures to create phase domains, or membrane features that they could study. Tall lipids, red in illustrations in the paper, melted at higher temperatures than short lipids, represented by green. The researchers melted the lipids together, then placed them on a surface to cool and separate into splotchy patterns. This is the system that Longo had developed for other purposes in earlier work.

"The tall guys tend to like each other and the short guys tend to like each other and shorts and talls tend to separate," Boxer said. "Like likes like."

Kraft had labeled the short and tall lipids with isotopes-elements that compare exactly in chemical properties because they have the same number of protons but that differ in mass because they have a different number of neutrons. Isotopes do not change the chemical structure of the molecules that they label. In contrast, the dyes commonly used in biological studies for labeling change the structure of the molecules to which they attach and can alter their behavior. Moreover, dyes do not allow scientists to analyze membrane compositions, because scientists see only what they've labeled.

"If you want to look at lipids certainly, lipid organization, you need some other way [than dyes]," Boxer said. "You'd want to have a way that's specific, that tells you which component is which. I'll put on my chemist hat now. When a chemist says, 'I know what's there,' he or she means, 'I know chemically what's there.' I know that this is cholesterol or this is sphingomyelin or this is some other molecule. You need a way to do analytical chemistry, only with extreme sensitivity, because we're not talking about very many molecules."

The answer is isotopes. The NanoSIMS 50 can detect extremely small differences in mass among molecular fragments labeled with different isotopes. And the machine has five detectors, so scientists can look at five different isotopically labeled molecules at a time. The picture from each isotope is overlaid to produce a composite image that provides rich, mass-specific information about chemically what's in that cell membrane.

Boxer compared the concept to learning about people sitting in chairs in a room to the organization of individual molecules in a membrane. "If you look at the room, you want to be able to see not just the chair but also what kind of chair it is... and I want to see who is nearby, and that chair over there is empty... I'd like to know that you're sitting in a chair and that that chair is different from that [other] chair. And that's what's

been missing. We just don't have analytical tools that give us this combination of sensitivity, analytical information and spatial resolution."

For the Science study, Boxer and colleagues made a spherical lipid vesicle to model a cell membrane, placed it on a small silicon wafer to make the lipid bilayer flatten into two dimensions and organized the flat membrane with a pattern of chrome grids to provide "landmarks" on the surface. That system let the scientists track how lipids moved and measure how many of each type of lipid resided in an area.

"We're looking for lateral organization of some sort believed to be present in membranes," Boxer said. "There is now no really good way to measure it. There's got to be something that makes one part of a cell different from another part of a cell. But the forces that are responsible for segregating components, or compartmentalizing components in cell membranes, simply are not known."

## Lipid rafts

Scientists have long known that certain proteins get pieces of grease stuck on them-become "prenylated," in chemical parlance. The addition of a hydrophobic group to a protein forms a lipid anchor that attaches the protein to the lipid membrane. Associations of different proteins in a section of compositionally distinct fatty membrane have been called "lipid rafts."

Just as a raft is a bunch of logs associated because a rope binds them together, allowing it to perform the function of floating, proteins associated with other proteins on a lipid raft may perform functions, Boxer said, though evidence for this is limited. These rafts may serve many functions such as reacting to stress, conferring immunity through antibody response, adhering to other cells and countering bacteria and toxins.

"Many proteins live in three dimensions for part of their life and in two dimensions for part of their life," Boxer said. "They go back and forth. When you're in two dimensions, your chance of bumping into something else is a lot higher than when you're in three dimensions. This is the idea of the rafts. You get one of these pieces of grease stuck on you, and now you become associated with the membrane and you find yourself in one of these rafts, whatever they are, and now you meet your friends, and the result is specialized function. It's an organizing principle, if you like, in an otherwise fluid environment."

Source: Stanford University