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Cell Membrane Mystery

ACS Meeting News: Lipids may not be arranged the way scientists thought, imaging mass spec data suggest

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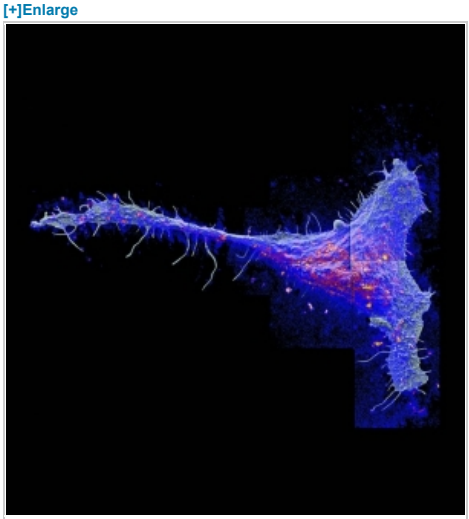

CLUSTERS
NanoSIMS imaging shows sphingolipid domains (red) in mouse fibroblast cells. The cell is about 65 µm across.
Credit: Kevin Carpenter

Though they're the most obvious part of cells, cell membranes have been a frustrating puzzle for scientists. Despite years of gathering information on the lipid and protein composition of membranes, biologists and chemists still haven't fully established how the components are organized. That's partly because the membranes are fluid, so the structure is constantly changing in subtle ways. One thing scientists agree on is that some level of localized organization must be taking place.

Biologists have posited the lipid raft hypothesis as a way to describe these localized structures. The rafts are proposed to be domains within the membrane that are enriched in cholesterol and a class of lipids called sphingolipids. These rafts seem to be crucial structures, playing a role in essential functions such as cell signaling. But without a clear picture of what the domains look like, scientists can't truly understand those functions.

The best evidence for the existence of lipid rafts relies on indirect measurements of the membrane components, rather than on viewing the components themselves. Furthermore, the central questions that remain in understanding them concern how the multiple components mingle in the same spot, or colocalize, which is particularly tough to see.

Two studies reported in the Division of Colloid and Surface Chemistry at the American Chemical Society national meeting in New Orleans earlier this month provide direct measurements—and evidence that the conventional view of lipid rafts might need tweaking. These insights came from using an imaging technique called nanometer-scale secondary ion mass spectrometry (nanoSIMS) to visualize synthetic and real lipid membranes.

In nanoSIMS, a beam of primary ions such as Cs^+ bombards a surface, producing secondary ions from the sample that can be measured by mass spectrometry. Images are formed by moving the ion beam across the sample surface and collecting mass spectra at many spots. The strength of the method is its ability to directly measure membrane composition at high spatial resolution.

NanoSIMS is not without its drawbacks. It requires a high vacuum, which is not compatible with aqueous membranes. In addition,

nanoSIMS detects smaller pieces of the membrane components after they have been fragmented rather than the intact molecular ion, so that piece of information is lost. This can be troublesome in the case of lipidsbecause the same ion fragments are often produced by different lipids. Researchers circumvent this problem by changing the isotopes of selected atoms in the specific molecules they want to see. The abundance of the substitute isotopes relative to the original natural isotopes in the sample helps pinpoint the location of the components.

In New Orleans, **Steven G. Boxer**, a chemistry professor at Stanford University, reported how his group, including postdoc Mónica M. Lozano, acquired chemical pictures of a four-component synthetic model membrane system to determine the chemical organization of lipid membranes (*J. Am. Chem. Soc.,* DOI: **10.1021/ja310831m**). Most other model membrane systems have included no more than three components.

In a separate presentation, **Mary L. Kraft**, an assistant professor of chemical and biomolecular engineering at the University of Illinois, Urbana-Champaign, reported having gone even further and analyzed real cell membranes (*Proc. Natl. Acad. Sci. USA,* DOI:**10.1073/pnas.1216585110**). Kraft collaborated on the project with **Joshua J. Zimmerberg** of the National Institutes of Health and **Peter K. Weber** of Lawrence Livermore National Laboratory.

Boxer's model membrane included ^{15}N-labeled dioleoylphosphatidylcholine, DOPC, detected as the $^{12}C^{15}N^-$ ion fragment; ^{2}H-labeled sphingomyelin, detected as $^{12}C^{2}H^-$; ^{13}C-labeled cholesterol; and ^{19}F-labeled ganglioside G_{M1}. The labeled ganglioside was obtained from collaborator Krishna Kumar, a chemistry professor at Tufts University, and his graduate student Zhao Liu.

Boxer and his coworkers used the labeled components to form cell-like vesicles, which they deposited on a silica-modified silicon surface, Boxer explained. They then heated the membranes to anneal them and rapidly freeze-dried the samples to preserve the membrane arrangement so it could withstand the high vacuum of the mass spectrometer.

They then used nanoSIMS to determine the distribution of isotope-labeled components and their colocalization in the membrane. Boxer said they found nanometer-scale domains of sphingomyelin within larger domains of ganglioside clustered with cholesterol. The ganglioside and cholesterol are usually considered to be good indicators for lipid rafts, so seeing them together is not a surprise.

What was a surprise was that sphingomyelin didn't cluster with the other two components but instead floated in its own domains within the larger domain. So the composition of domains they observed suggests the membrane features that biologists call rafts may not be made of the exact components as previously assumed.

Another interesting finding in Boxer's work was the heterogeneous composition of the vesicles. In model systems, the usual assumption is that the ratio of vesicle components to each other reflects their ratio in the starting mixture. But the nanoSIMS data suggest that assumption may not be valid. For example, the membranes had lower DOPC levels than expected.

"Forget how the components are distributed locally on some length scale," Boxer said. "Is the composition itself correct? The answer is going to be distressing to a lot of people."

But some people are skeptical of the findings. **Gerald W. Feigenson**, a membrane biophysicist at Cornell University, wonders about the results because no phase diagram for a four-component system exists with which to test the accuracy of the compositions Boxer found. "Having such a phase diagram would be very helpful for interpreting the data," he said, "because the relative-area fractions of phase domains could be determined."

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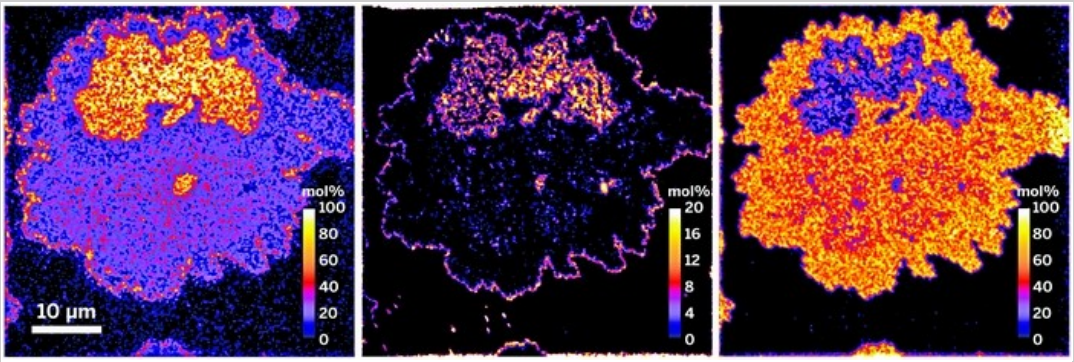

TOGETHER, OR NOT
In these nanoSIMS images of a four-component model membrane, cholesterol (left) and ganglioside G_{M1} (center) localize in the same region, whereas sphingomyelin (right) does not. Orange represents high levels of the components, and blue or black represents low levels.
Credit: *J. Am. Chem. Soc.*

In addition, the nanoscale domains-within-domains could be strongly influenced by the rate of the freezing protocol, Feigenson said. For example, the Boxer technique for freezing the sample is fast but perhaps not fast enough to capture the instantaneous composition of the domains, he noted.

“I would conclude that this study has discovered a surprising phase behavior of lipids that are tightly bound to a silica surface, a finding that might or might not be closely related to the phase behavior of bilayer membranes surrounded by an aqueous phase,” Feigenson said.

Others are more enthusiastic. **Marjorie L. Longo**, a membrane engineer at the University of California, Davis, called the Boxer group’s work “an important result because they were able to distinguish the composition at such high resolution.” Longo suggests that a good test would be for other researchers to see whether they observe similar trends in the same four-component system using different analytical methods.

The results on actual cell membranes by Kraft and her coworkers also turned up some surprises. Her team started out fully expecting its nanoSIMS experiments to verify the conventional raft model in cultured mouse fibroblast cells.

The researchers tagged the sphingolipids by feeding the cells ^{15}N-labeled sphingolipid precursors. They chose labeled precursors that aren’t found in the biosynthetic pathways of other lipids, which allowed them to be more certain of where the label would end up. In addition, they labeled all the membrane lipids, including the sphingolipids, with ^{13}C-labeled fatty acid precursors.

To prepare the samples for nanoSIMS, they chemically fixed them using glutaraldehyde and osmium tetroxide to prevent the components from diffusing when the samples were dehydrated. By using nanoSIMS, they found that sphingolipids clustered in domains.

Previous research in the field suggested that cholesterol is involved in domain formation. Kraft’s data support that idea, but the researchers gained new insight by giving the cells ^{18}O-labeled cholesterol. They found the labeled cholesterol distributes relatively uniformly throughout the cell membrane, including in the sphingolipid domains. That is, the cholesterol does not preferentially associate with the sphingolipids.

When the researchers chemically treated the membranes using methyl-β-cyclodextrin to deplete the cholesterol, the cholesterol that remained was still relatively uniformly distributed, but there were fewer sphingolipid domains.

Kraft and her team then disrupted the cell’s cytoskeleton—its internal scaffolding. They used the drug latrunculin A to depolymerize the protein actin, a key part of the cytoskeleton. Without an intact cytoskeleton, the sphingolipids no longer formed domains.

“Our results are consistent with the idea that cholesterol abundance does affect membrane organization,” Kraft said. “It’s just not due to preferential interactions between cholesterol and sphingolipids. Cholesterol is affecting something that’s affecting something else—I don’t know how many things—that eventually affects the sphingolipid organization in the cell membrane.”

But Feigenson has doubts about this method as well. For example, the researchers allowed 30 minutes for glutaraldehyde cross-linking to purportedly trap the domains, he said. During that time, membrane components could still diffuse and form larger domains than found in the cell. “I would not trust a process that takes many minutes to trap tiny domains,” Feigenson added.

Boxer’s and Kraft’s experiments are part of a trend in which scientists are looking at increasingly complicated systems, such as those that might form lipid rafts, and realizing there’s a big difference between a three-component membrane of lipids you can buy from a lab supply company and a 100-plus-component membrane in a real cell, Longo observed. “As more techniques are brought to bear on cell membranes, we’ll find that they’re much more complicated than we imagine—as it always is with real biological systems.”

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