Viewing dye-packed vesicles causes them to explode

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It's a long-standing question: Can just the act of observing an experiment affect the results? According to a new study by Rockefeller University scientists, if the experiment uses a fluorescent dye called acridine orange, the answer is a resounding "yes."

Cells use a process called exocytosis to deliver membrane-bound vesicles full of proteins, neurotransmitters and other molecules to their outer membrane and beyond. Among other things, these little chemical packets are vital for cell-to-cell communication.

So researchers interested in better understanding exocytosis have been using acridine orange to label the vesicles in an attempt to observe the process. Because the dye gets trapped in multiple vesicles and increases its fluorescence upon release from the vesicles, the resulting characteristic flash has been considered a hallmark of exocytosis imagery.

But research published in the *Proceedings of the National Academy of Sciences* by Sanford Simon, head of the Laboratory of Cellular Biophysics, and research assistant professor Jyoti Jaiswal, shows that the characteristic acridine orange flash is not the exclusive result of exocytosis at all. Instead, light from the microscope was also causing vesicles to burst in a process known as lysis. "Not only do the dye molecules concentrate in vesicles but, at higher concentrations, it's known that light can cause acridine orange to lyse them," Jaiswal says. Just looking at the vesicles through a microscope has the potential to

disrupt lipid membranes.

While doing an experiment in 1992, Simon used acridine orange to study exocytosis and saw just what he expected. “For 20 to 30 seconds, I was ecstatic,” he says. “But then I realized that it didn’t necessarily mean we were looking at exocytosis. We were possibly just creating photodamage.” His lab has since used other means to study the process.

Then a recent series of papers, in which the authors used acridine orange to explore calcium-triggered exocytosis in astrocytes, aroused Simon and Jaiswal’s suspicions. Astrocytes are small, star-shaped glial cells that are part of the neural system’s support network; because their role in brain physiology and neural regulation is only just beginning to be addressed, understanding exocytosis in these cells is particularly important. So the Rockefeller researchers did their own experiments with acridine orange. They found that when they looked through the microscope they could, indeed, see bright flashes of dye that looked as if the vesicles were fusing with the cell’s plasma membrane. But when they moved the microscope field to a different area of the cell, they saw another flurry of fireworks — proof that light from the microscope was prompting the acridine orange-filled vesicles to lyse.

This puts the prior acridine orange–exocytosis experiments into question, Jaiswal says. “Using acridine orange means that the onus is on the person doing the experiment to prove that what they’re seeing is fusion.” And that was precisely what he and Simon went on to do. In order to differentiate between exocytosis and imaging-induced lysis, Simon notes that it’s important to quantify the results. So the two researchers put together a combination of experimental and mathematical techniques that could then be used to discriminate between vesicles that are lysing from those that are fusing with the membrane. By fluorescently labeling the vesicle membrane protein with a different, nondisruptive marker, and quantifying how the particles dispersed, they

were able to distinguish between the two processes and found that calcium-triggered exocytosis does occur in astrocytes — just not to the exaggerated degree it had appeared using acridine orange.

In fact, Jaiswal and Simon were even able to pin down at least one of the astrocyte organelles that can undergo this kind of exocytosis: lysosomes. Exocytosis of these membrane-bound compartments is already known to play a role in immunity and healing wounded cells, so the new findings provide another avenue for researchers to pursue. “There’s already a body of literature on lysosome exocytosis and its roles, and we’ve looked at how it’s regulated,” Jaiswal says. “Now, all that literature has become relevant to astrocyte biology, too.”

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