New microscope unlocks the cell's mysteries at the molecular level

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(PhysOrg.com) -- Among science's "final frontiers," one of the most difficult to cross has been looking into the molecular-level workings of living cells. Now, a University of Massachusetts Amherst physicist has built an instrument to do just that and is beginning to uncover secrets such as how enzymes regulate various cell functions.

Jennifer Ross built a microscope she calls Single Molecule TIRF, for total internal reflection fluorescence, that is much brighter than commercially available instruments and has the remarkable ability to see and photograph single molecules in real time.

An image from the TIRF instrument from one of Ross and colleagues' recent studies of the enzyme katanin was recently featured on the cover of *Biophysical Journal*, accompanying their article reporting that they have for the first time seen and recorded video of an enzyme cutting microtubules. This accomplishment is a key to understanding basic microtubule function and what goes wrong in diseases related to their malfunction.

As the name suggests, microtubules are strong, hollow tubes about 25 nanometers in diameter that form bundles to provide structure to a vast variety of cells from plants to humans. In plants, they direct cellulose deposition to give plants rigidity, Ross notes. In humans, nerve axons cannot function properly without long stabilized microtubule bundles to support their extended structure. Without them, nerve cells retract, causing neuromuscular diseases such as amyotrophic lateral sclerosis (ALS) or spastic paraplegia. Microtubules are also crucial in arranging materials inside cells during the two types of 2division, mitosis and meiosis.

Katanin is an enzyme that cuts microtubules in the middle or near either end, making it an important regulator in control of these molecular structures. "Think of microtubules as a bunch of lumber you want to use to build a house, but you have no way to cut the boards into the correct lengths. You need katanin to cut it," says Ross.

However, there's a long-standing lack of understanding of how this enzyme actually cuts microtubules, she adds. One reason is that it's very difficult to purify. Her postdoctoral research assistant Juan Daniel Diaz-Valencia has made "valiant" efforts to purify katanin for the series of experiments conducted at UMass Amherst in collaboration with cell biologist David Sharp of the Albert Einstein College of Medicine, Bronx, New York. When Diaz-Valencia successfully purifies katanin, "he stays up for two days to get data," she says.

In a recent series of experiments, her group not only documented katanin's cutting action but also discovered that the action is concentration-dependent. They also established that taking katanin away from a cell results in a microtubule buildup that chokes the cell inside like a logjam. "No one has characterized it quite as well as we have," Ross points out. "Because studying it in bulk solution is not helpful. It's essential to have the Single Molecule TIRF microscope to visualize exactly what's going on."

For these studies, the researchers worked with a preparation of purified pig brains, rich in microtubules, adding katanin labeled with a fluorescent tag to visualize the mechanics of how the katanin "snipping" complex works. Through the TIRF microscope, they take videos with a very sensitive camera that can see single light particles.

After a three-minute control period passes at the start of each experiment to make sure the microtubules are not destabilizing on their own, which provides a control for each session, the researchers add purified katanin in different concentrations. By measuring the brightness of the fluorescent tags, they can count the number of molecules present more precisely than ever before, Ross notes.

"What we've found is that katanin is constantly breaking down and reforming as it is being used to cut the microtubules," she adds. "We now know that it's constantly recycling subunits, as if you're continually replacing wheels on your car as you're driving along." But while the protein is chopping the microtubules, it's also destroying the experiment, so after 20 minutes they must start a new one.

Ross and colleagues are already moving on to next steps, building an even more powerful new microscope with more capabilities using National Science Foundation funding. They will be turning their attention next to a relatively unknown enzyme called fidgetin, named

after a mouse strain with a tremor that twitches its head back and forth rather than the usual up and down. The animals, first noted in the 1940s, suffer from a mutation in fidgetin production causing this unusual tremor.

Only in the past decade have biologists begun to examine fidgetin's function at the molecular level, Ross says. "We find that this protein is unique, it's very different from katanin and it regulates bone formation," she explains. "Lack of it causes birth defects." She and colleagues are beginning work with developmental biologists including Dominique Alfandari in the UMass Amherst veterinary and animal sciences department to design further studies.

"We're seeing such unusual things, we can't afford to not look at it," she adds. "I will not be surprised if, in 10 years, we identify numerous bone birth defects that are caused by the lack of this enzyme."

Provided by University of Massachusetts Amherst

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