Biophysicists unravel cellular 'traffic jams' in active transport

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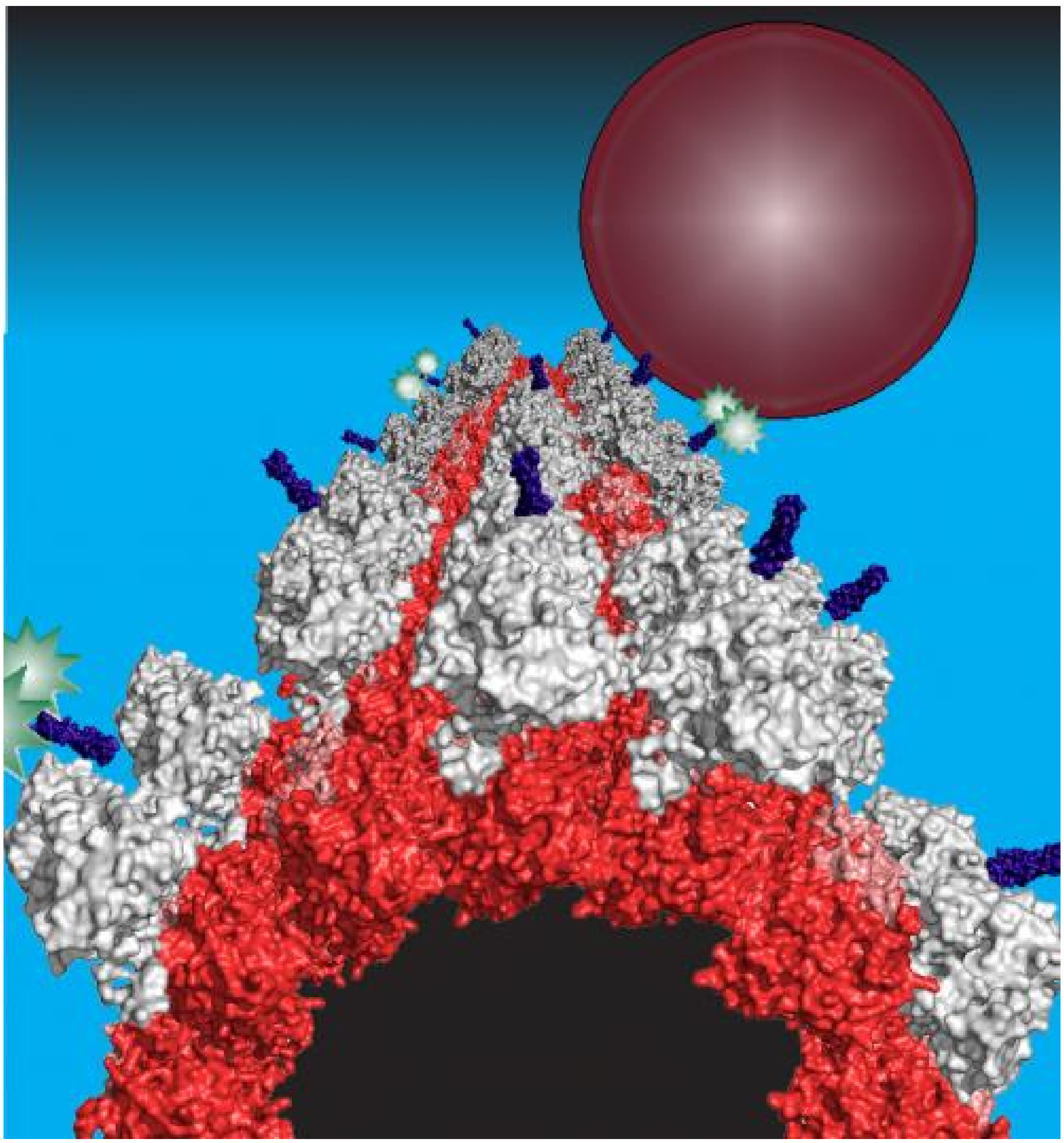

This image depicts motor protein traffic along a single microtubule highway. Much like vehicular traffic in real life, kinesin motor traffic reduces the velocity of single motors. Multi-motor cargos, such as the quantum dot depicted, can stay attached to the microtubule much longer because they can add multiple motors. Credit: Leslie Conway and Jennifer Ross, UMass Amherst

UMass Amherst biophysicists, using a unique microscope, have improved upon earlier studies that used too-simple models not able to account for the densely crowded, dynamic conditions of a active transport in a real cell.

Inside many growing cells, an active transport system runs on nano-sized microtubule tracks that resemble a highway, complete with motors carrying cargo quickly from a central supply depot to growing tips or wherever materials are needed. In spite of the cell's busy, high-traffic environment, researchers know the system somehow works efficiently, without accidents or traffic jams.

Now a team of biophysicists at the University of Massachusetts Amherst, using a special technique and unique microscope, have improved upon earlier studies that used too-simple models not able to account for the densely crowded, dynamic conditions in a real cell. The new work, led by biophysicist Jennifer Ross, greatly advances understanding of how active transport proceeds smoothly, particularly in long cells such as neurons where it is vital to their survival. Findings appear in the current early online edition of *Proceedings of the National Academies of Science*.

Ross says, "What others learned from the simpler models and experiments gave insight into how single motors work, how load affects velocity, and how single motors are stopped by static objects such as microtubule-associated proteins or intersections. But so many questions remained, such as what happens in a high-traffic with many other motors? How can single motors and cargos with many motors efficiently maneuver?"

With this work, she says, "We're getting closer to understanding the more complex, crowded environment of the cell and how large objects, like organelles, can be moved from the cell body in your spine out to your big toe along a neuron one meter long. Although previous studies thought that traffic would be a problem because it would cause motors to detach, we found it is not a problem for an organelle because there are so many motors. We found that the traffic would slow you down, but not enough to hurt any essential processes. It would take the organelle 12 days instead of six to travel a meter."

A key to Ross and colleagues' experimental system is a custom-built, single-molecule total internal reflection fluorescence (TIRF) microscope she built for her laboratory. It is much brighter than commercially available instruments and gives researchers the remarkable ability to see and photograph individual molecules in real time.

For this investigation exploring the effect of high traffic on active transport via microtubules, they also used quantum dots (Qdots), a nanocrystal of semiconductor material that fluoresces in different colors based on size, making them useful biological probes, Ross points out. "We use them as a stand-in, a model for common cellular cargo such as a mitochondrion, after we discovered that they bind to multiple cellular kinesin motors. If individual motors are like cars, Qdots are a bus," she adds.

The transportation analogy works remarkably well for understanding the molecular-level active transport system, says Ross. The multi-lane nano-scale microtubule system in a cell is like a multi-lane highway along which essential cargo is moved. An organelle needed at the growing tip floats around until it finds a microtubule. Kinesin motors with two "feet" that fit neatly into the tracks then latch onto it and "walk" along toward the goal in prescribed footprints.

But a basic problem for the motors is they cannot change lanes. If they come upon a slow-moving or stopped motor in the track, all traffic stops in that lane and cargo may be released. This led earlier observers to assert that stalled protein on the microtubules, a "traffic jam," poses a threat to active transport. Yet Ross says evidence doesn't support this view; somehow cells find a way to use active transport smoothly and successfully.

In the UMass Amherst experiments, she says, "We're trying to find out how cells do this, work without accidents or stalls. We set up experiments in which we kept adding motors to see what happens in a high traffic situation." An unusual quality of the Qdots, Ross adds, is that they attach to multiple motors and move along all lanes of traffic at once.

The researchers use three colors for labeling the Qdots, 10 percent of the motors and the microtubules, then take videos in real time to measure how far the single-track motor moves in a given time compared to how far the Qdot goes. They also measured how fast each component moved and how much time each spent on the microtubule.

"We were surprised at first because the Qdot could move eight or 10 microns along the track in these high-traffic situations when a single motor could almost not move at all. Then we realized the Qdot had multiple motors attached and further, it could take on or let go of motors while still holding onto the other parts of the track. They might have 10 motors bound with 20 feet on several tracks. So even if a single motor gets hung up, the cargo can still move," Ross explains.

"Interestingly, the Qdot and single-motor velocities were identical, so they didn't go any faster, they just stayed on longer and it enabled them to go farther. We have showed that having multiple motors is one way a cell can handle high traffic situations in active transport."

"We've shown that high traffic is not an issue. It slows things down but it does not stop transport. I think this is a big discovery. Others before us have said that traffic could bring it all to a halt, but it turns out that if you have multiple motors it would solve this problem."

Provided by University of Massachusetts Amherst