

Full Tilt Transport Electron tomography's three-dimensional images give surprising insights into cell trafficking.

LAVA LAMPS SEEM SIMPLE AT FIRST, BUT STARE AT one for a while and the complexity behind how the colored blobs stretch, tangle, and pinch off becomes evident. Cells use similarly complex and dynamic structures for transport, according to HHMI investigator Pamela Björkman of the California Institute of Technology. New images from her lab reveal ornate structures cells use to shuttle proteins that help a newborn fend off infections.

Developing fetuses and newborns don't have fully formed immune systems. Instead, the mother provides protection by donating her antibodies. A newborn absorbs them from its mother's milk with the help of a protein called FcRn. The protein sits in the outer membrane of intestine cells and grabs antibodies as they pass through the gut. A sac called a vesicle pinches off from the membrane, carries FcRn and antibodies to the other side of the cell, and dispenses antibodies into the baby's bloodstream.

Björkman has devoted years to studying high-resolution details of the protein's shape by shining x-rays through FcRn crystals. Because making crystals out of membrane proteins is tricky, she has been able to study only the portion of the protein that pokes out from the membrane—the part that clamps down on antibodies. She knew, however, that the protein behaved differently when it sat in a membrane. Plus, the x-ray method probes the protein in isolation, and Björkman wanted to know how it worked in the intestine.

To learn more, she turned to a technique called electron tomography. The method builds on electron microscopy (EM), in which beams of electrons pass through a tissue sample to create a detailed two-dimensional image. In electron tomography, multiple images are captured while tilting the sample at different angles relative to the electron beam. The images can then be analyzed and combined into a three-

dimensional picture. Björkman's team developed antibodies tagged with gold particles so that FcRn was visible when it grabbed an antibody.

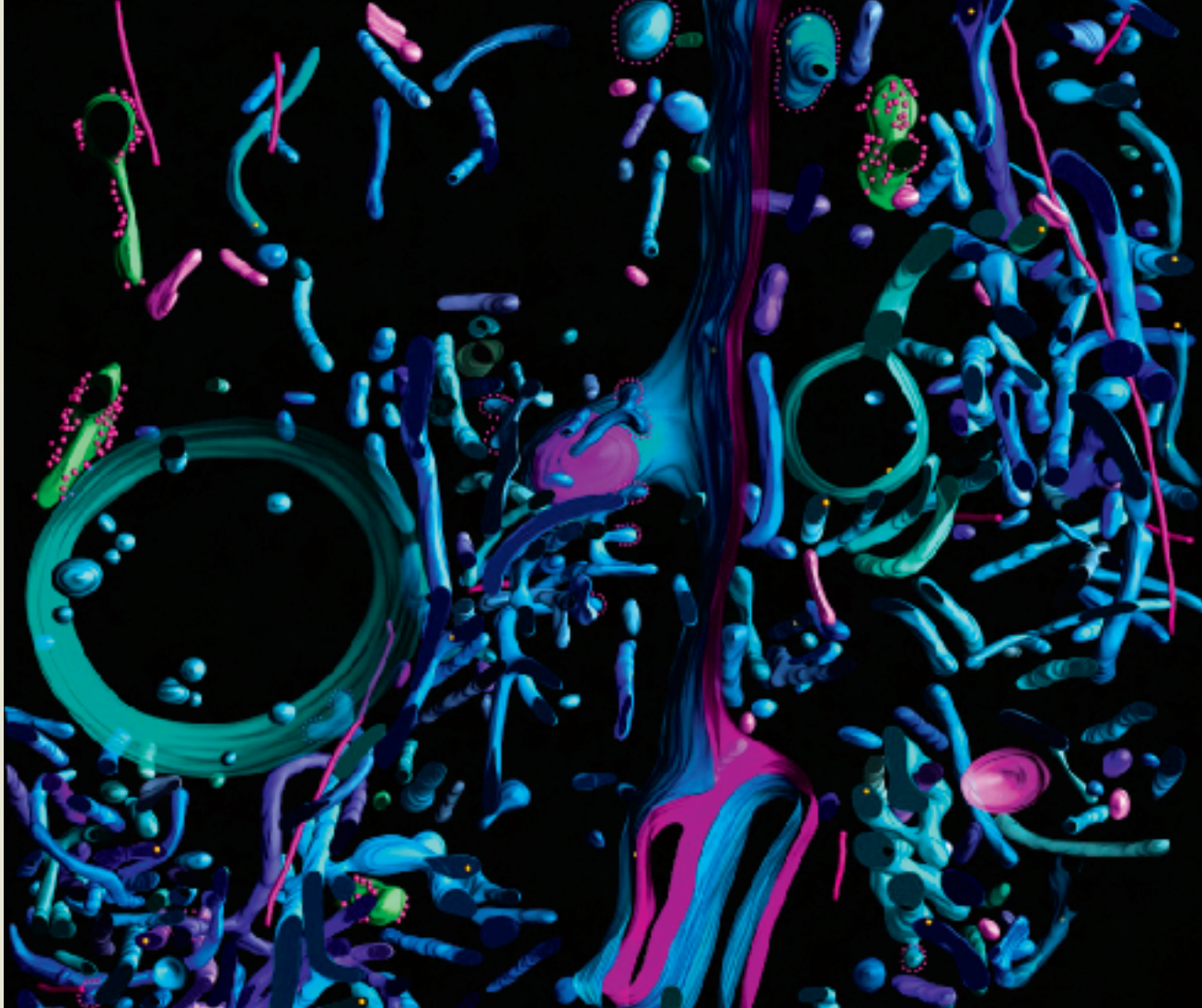
What they saw shocked them. For example, they found amalgams of FcRn and antibodies in enormous balloons carrying lots of vesicles. Normally, these big balloons carry proteins to the cell's recycle bins. But other molecules present in the structures suggested that they helped transport antibodies across the cell, not dispose of them. The structures also formed long tubes and broke off into small vesicles that—apparently—convey antibodies through the cytoplasm.

An efficient trucking system might involve a small number of vesicles specialized to carry just FcRn, so Björkman expected to see a few vesicles containing a lot of FcRn and antibody. Instead, cells had a lot of vesicles holding only a little of the cargo. Moreover, the vesicles weren't neat spheres. They were “looped

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and bent, and twisted around each other. They almost tied themselves in knots,” says Björkman. “It was a tangled mess I wasn't expecting.” Previous results from two-dimensional imaging had suggested a more orderly grouping of small vesicles, where vesicles typically travel across cells on protein tracks. But in Björkman's observations few vesicles were lined up with these tracks. She suspects that once the longer tubes expunge the smaller vesicles, those small vesicles move randomly through the cell until they collide with the membrane.



By tagging antibodies with gold particles, Pamela Björkman's group has visualized, using electron tomography, how a newborn rat absorbs these antibodies from its mother's milk and transports them within a cell. The resulting images reveal several surprises about key transport vesicles (blue) among other cellular components (various colors).

The delivery step also bore a surprise. As expected, vesicles arrived at the blood vessel side of the cell and fused with the membrane to release their antibody load. But the vesicles also carried the tell-tale hexagons and pentagons of clathrin, a molecule that helps form vesicles by constructing a cage around them. Conventional wisdom holds that vesicles have to shed this cage before they deliver their cargo, so Björkman was surprised to see clathrin there. The observation might reveal a new mechanism for releasing cargo, says Björkman. For instance, vesicles might shed only a small part of their clathrin coating to quickly jettison antibodies and immediately return to pick up a new load.

Björkman is now after a more detailed view of antibody transport, employing other methods to witness transport in action. “In EM, everything is in a vacuum; nothing is alive,” says Björkman. “But in fluorescent live imaging we can take 5 frames per second and watch vesicles move.” Gazing at the mysteries those movies hold could provide action-packed evidence for some of Björkman’s speculations.

■ -R. JOHN DAVENPORT



WEB EXTRA: Visit the *Bulletin* online for a slideshow of Björkman's electron tomography images.