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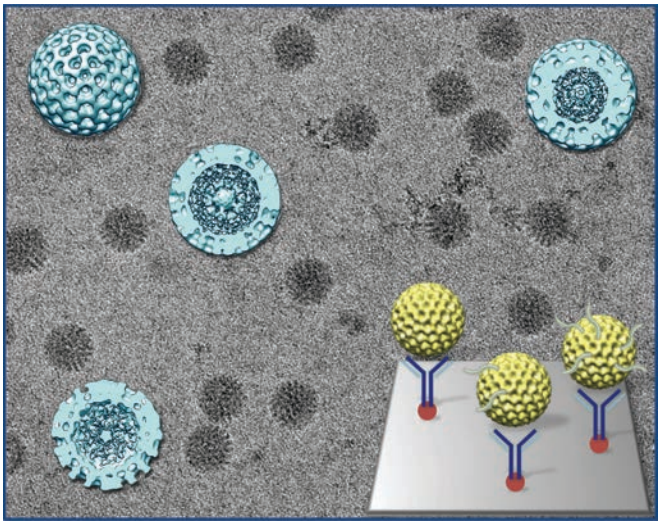
PRESS RELEASE

Structural insights into the inner workings of a viral nanomachine



Captured rotavirus double-layered particles (DLPs) in the midst of producing RNA. The schematic represents an EM Affinity Grid (gray square) coated with adaptor molecules (red and dark blue) that anchor active rotavirus DLPs (yellow) to the Affinity Grid. Cryo-Electron Microscopy (EM) image of actively transcribing DLPs reveals RNA strands (gray strands) emerging from the virus capsid. Three-dimensional image reconstructions of DLPs (light blue) that actively produce RNA reveal strong density within the viral core. Diameter of each reconstruction is ~80 nm. (Credit: Deborah F. Kelly, Virginia Tech Carilion Research Institute, Virginia Tech.)

Researchers at the Virginia Tech Carilion Research Institute (VTCRI) are using new nanoscale imaging approaches to shed light on the dynamic activities of rotaviruses, important pathogens that cause life-threatening diarrhea in young children. Once a rotavirus enters a host cell, it sheds its outermost protein layer, leaving behind a double-layered particle (DLP). These DLPs are the form of the virus that produces messenger RNA molecules, which are critical for launching the infection. Researchers, Deborah Kelly, Ph.D. and Sarah McDonald, Ph.D., both Assistant Professors at VTCRI, acquired molecular snapshots of rotavirus DLPs, in the midst of producing viral RNA, using cryo-Electron Microscopy (cryo-EM). The team performing the work also included third year medical students, Joanna Kam and Andrew Demmert, of the Virginia Tech Carilion School of Medicine, and post-doctoral fellow, Justin Tanner, Ph.D.

To get the best possible view of the nanoscale details of active rotavirus DLPs, Kelly developed a technique that permitted visualization of changes in the outermost shell. In conjunction with novel computational approaches, the scientists were also able to detect the internal features of the DLPs, which had not been previously observed. Interestingly, the internal DLP

features changed in a manner that corresponded to observable differences in levels of viral messenger RNA production. These findings provide new structural insights into the mechanics of rotavirus RNA synthesis, which may in turn provide information about how this viral process takes place upon host cell infection. The results appear in the latest edition of the journal *TECHNOLOGY*.

"What's remarkable about this study is that we were able to see different levels of complexity inside the DLPs that correlated with viral RNA synthesis," said Kelly. "When viruses were active, their external structures moved dynamically, in a way that became less organized. While, at the same time, strong features within their internal cores become more prominent."

A key innovative approach used by the Kelly laboratory has provided a chance to view a wider spectrum of viral structures. By examining the DLPs attached to antibodies on a stable grid surface, researchers were able to view the nanomachines cycling through their natural processes.

Kelly and McDonald also used a new computer algorithm to categorize the DLPs, independently, which avoided user-bias in the experimental calculations. The statistical-based computational approach classified the samples based on levels RNA production. The results clearly showed that rotavirus DLPs with a less organized outer protein layer had more solid details in their internal cores. These DLPs were also found in the cryo-EM images to be near more RNA strands.

"For many years scientists have been concerned with higher-resolution results and have not paid close attention to the subtle diversity that exists in virus samples," said McDonald, who is also an Assistant Professor of Biomedical Sciences and Pathobiology at the Virginia–Maryland Regional College of Veterinary Medicine. "But that diversity may be indicative of how viruses actually function inside cells. They are not static, but dynamic in nature."

"It's a little counterintuitive," said Kelly, who is also an Assistant Professor of Biological Sciences in Virginia Tech's College of Sciences. "You would imagine that, if biological parts were moving around, then features would dissipate. When these rearrangements occur in such a confined space, however, it may potentially lead to a higher level of organization. And the coordinated changes on the outside of viruses seem to enable these processes."

According to Kelly, these results give new insight into the RNA synthetic processes of rotavirus and may prove useful in our understanding of viral biology in general. Improving our understanding of the inner workings of rotavirus, she added, might also provide new targets for the development of treatments for viral-induced diarrheal diseases.

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