

VIRAL PHYSICS LESSONS: Simulations Offer Novel Insights

By Kristin Sainani, PhD

Zooming in on a virus reveals a physical marvel. It can stuff a genome into a confined space (a protein casing called a capsid). It can eject its genome rapidly and fluidly into a cell. And it can coat itself in a piece of host cell membrane (a lipid envelope) to avoid detection by the host's immune system. To better understand how viruses per-

could lead to novel ways of crippling these creatures as well as ways to engineer viruses for therapeutic purposes.

Spring-Loaded DNA

To cram their genetic material inside a capsid, DNA viruses employ some of the most powerful molecular motors in nature.

out at the moment of infection, a process called DNA ejection. "We suspected that there is a one-to-one relationship between the way the DNA is packaged by the motors and the dynamics of ejection," says **Murugappan Muthukumar, PhD**, professor of polymer science and engineering at the University of Massachusetts, Amherst.

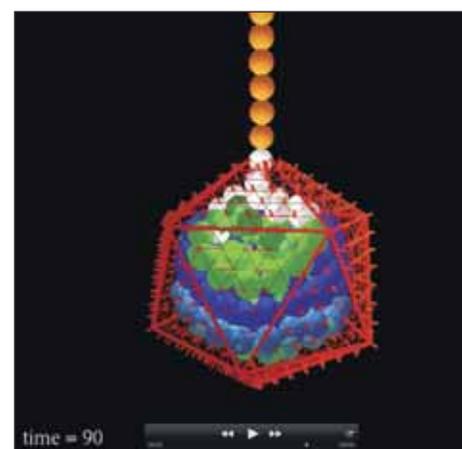
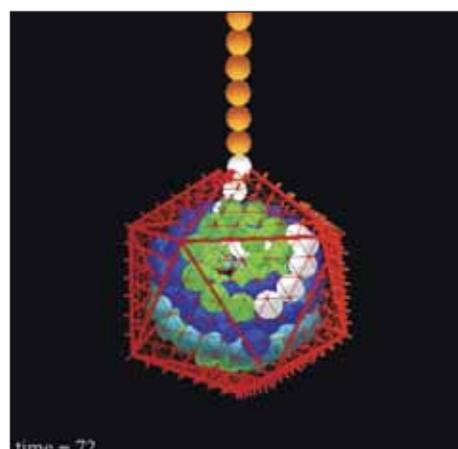
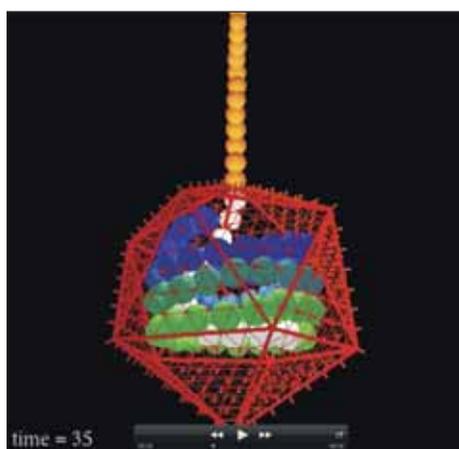
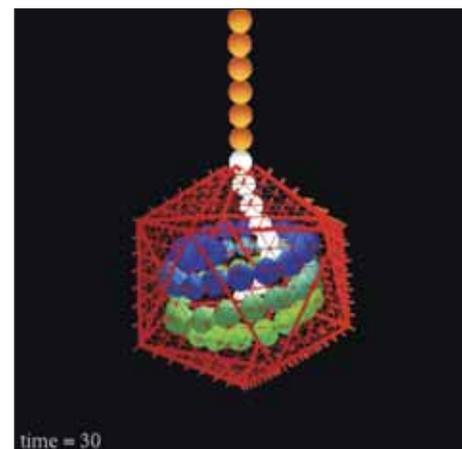
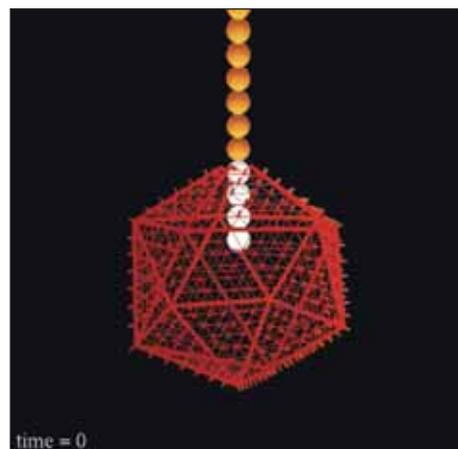
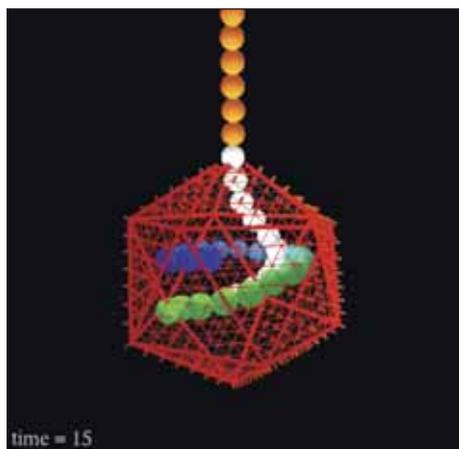
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form these parlor tricks, some scientists are turning to physics-based computer simulations. These methods are revealing some unexpected vulnerabilities in viral design that

It takes a lot of energy to bend the stiff double helix and overcome repulsive forces between strands. Once squished inside the capsid, the genome waits ready to spring

His team explored the connection between packing and ejection in bacteriophage in a 2013 paper in *Biophysical Journal*.

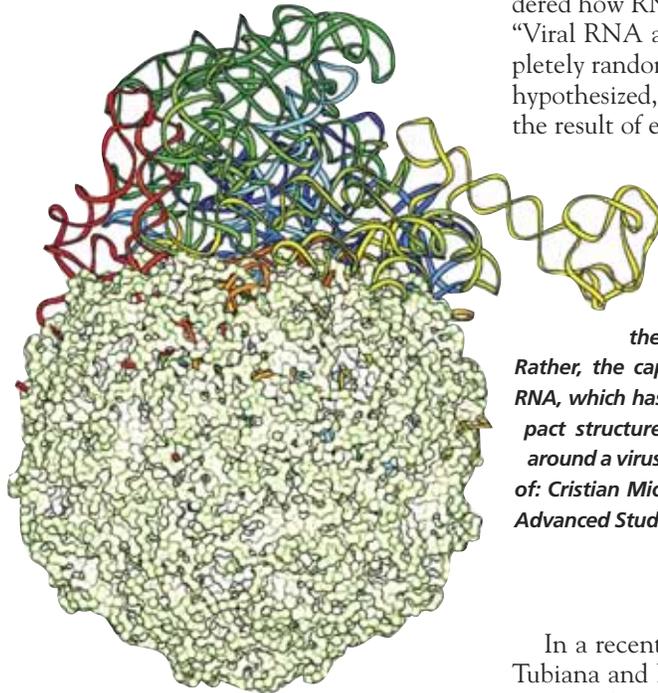
Muthukumar's team modeled DNA as



These screenshots show the progress of a DNA viral packing simulation from initial entry of the DNA to near completion. In repeated simulations, the researchers found that DNA organization after packaging varied and also depended on motor force. Courtesy of: Murugappan Muthukumar.

...Therapies could be developed to speed up the molecular motor enough to make packing more disorderly, causing the DNA to become stuck in the capsid. Or treatments might mess with the DNA inside the capsid, preventing it from achieving an effective exit path, Muthukumar says.

a wormlike chain of charged beads on a string. In their simulation, the motor stuffs the rope-like molecule through an opening in the capsid; after packing is complete, the DNA is allowed to spontaneously eject from the same portal. As experimentalists had previously observed, they found that the motor frequently stalls during packing. Experimentalists have blamed the pauses on motor choppiness,



Single-stranded RNA viruses don't require a motor to pack their genomes into the capsid.

Rather, the capsid self-assembles around the RNA, which has evolved to have an extra-compact structure. Here, capsid proteins gather around a virus's single-stranded RNA. Courtesy of: Cristian Micheletti, International School for Advanced Studies.

but Muthukumar's team showed that the DNA chain sometimes bunches up, and takes time to relax and make room.

They also showed that packing occurs slightly differently every time and is more variable and disorganized at higher motor speeds. The more ill-ordered the packing, the more inconsistent the ejection kinetics. "We watched each DNA monomer as it was coming out. We could see whether it was wrapping around, going in the wrong direction, or going in an orbital-like movement." They found that if DNA approached the exit portal at the wrong angle, it would become jammed, causing ejection to pause as the molecule straightened out. A 2014 paper in *PNAS* confirmed their predictions with experimental data.

These observations could be exploited therapeutically. For example, therapies could be developed to speed up the molecular motor enough to make packing more disorderly, causing the DNA to become stuck in the capsid. Or treatments might mess with the DNA inside the capsid, pre-

venting it from achieving an effective exit path, Muthukumar says.

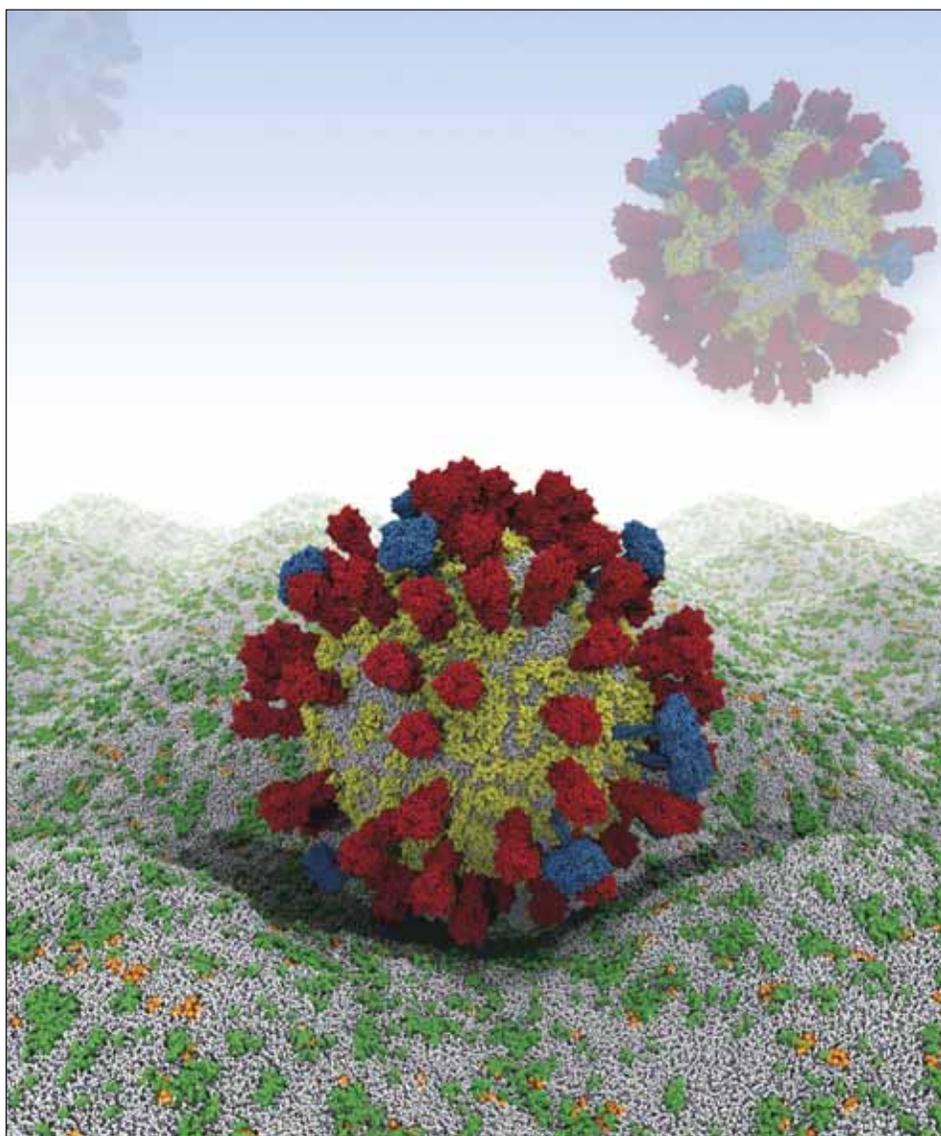
Self-Compacting Genomes

Unlike DNA viruses with their powerful molecular motors, many single-stranded RNA viruses pack their genomes with no help. Their RNA strands fold spontaneously into space-saving structures, and the protein capsid takes shape around them. Luca Tubiana, PhD, postdoctoral fellow at the University of Vienna, wondered how RNA viruses manage that task. "Viral RNA are more compact than completely random RNA," he observes. So, he hypothesized, perhaps their compactness is the result of evolution.

In a recent paper in *Biophysical Journal*, Tubiana and his colleagues put that question to the test. The team mutated the genomes of 128 different single-stranded RNA viruses *in silico* using only synonymous mutations, which do not change the amino acids encoded. Then they used software to predict how the RNA would fold. Strikingly, mutations in just five percent of the genome were sufficient to wipe out genome compactness. This was true even if they preserved codon bias (the fact that some codons occur more frequently than others) and restricted mutations to protein-coding regions. "This strongly indicates that this compactness is evolutionarily selected for," Tubiana says. These viral RNAs don't just code for specific proteins but also for physical shape, he concludes.

This knowledge could be useful in the design of antiviral drugs. "In principle, one may be able to destroy this physical compactness and therefore hinder the reproduction of the virus," Tubiana says. Plus, scientists are hoping to exploit viruses for therapeutics, such as engineering anti-cancer viruses. Understanding how capsid assembly works naturally will make this task easier.

“The details, the shapes of the models, come from experiments. But these experiments are largely static. With computer simulation, we can animate the system,” Reddy explains. “Basically, Newton’s laws of motion are being used to allow the atoms to wiggle and jiggle over time.”



In the future, scientists from the University of Oxford plan to build on their flu envelope model; for example, they may simulate its interaction with a host cell membrane, as pictured in this cartoon. Courtesy of: Heidi Koldsoe, University of Oxford.

The Physics of a Virus’s Shield

Many of the world’s deadliest viruses—including Ebola, HIV, and flu—are encased in a lipid envelope that shields them from the host immune system. Newly formed viruses steal pieces of the host cell membrane, into which they insert the viral spike proteins that will be used to latch onto new host cells. In a recent paper in *Structure*, scientists reported the first microsecond-timescale simulation of an influenza A virion’s envelope. Their goal: To gain insight into the flu virus’s biophysical behavior, says lead author **Tyler Reddy, PhD**, a postdoctoral fellow at the University of Oxford.

They based their models on abundant experimental data, including viral protein structure as well as the lipid composition of the envelope. “The details, the shapes of the models, come from experiments. But these experiments are largely static. With computer simulation, we can animate the system,” Reddy explains. “Basically, Newton’s laws of motion are being used to allow the atoms to wiggle and jiggle over time.”

His team first modeled the envelope as a lipid ball in a water droplet. Once the ball (vesicle) relaxed into equilibrium, they inserted viral spike proteins into the model and capped some lipids with sugars to fashion the glycolipids that account for 12 percent of the envelope. Though the model was coarse-grained to reduce computational demands, their five-microsecond simulations still took a year to run on a high-performance supercomputer.

The simulations revealed that the surface glycolipids slow down the movement of both spike proteins and lipids. “That makes sense because they’re basically these physical obstructions on the surface of the virus,” Reddy says. Low envelope mobility may help explain how flu viruses can survive in water for up to three years, he says. Reddy’s team also showed that the spike proteins don’t clump in the presence of glycolipids, which likely facilitates host cell binding but may also make the virus vulnerable to host antibodies.

Reddy’s team is working on a bigger simulation that incorporates both the flu virus and the host cell membrane to see how they interact. Eventually, they hope to use their model to probe how flu virions respond to different drugs.

These computer simulations reveal new insights into how viruses reproduce and spread. Beyond the implications for medicine, viruses’ slick anatomies also evoke wonderment. Muthukumar says: “Viruses are very beautiful objects.” □